Genetic variation in life history strategy and the responses of plant populations and communities to climate change

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Abstract

Climate change poses a major threat to biodiversity. Evolutionary responses may provide populations with a mechanism to adapt and persist through climate change. However, we still know little about the capacity for evolution in natural populations, or the genetic or ecological processes that constrain or facilitate responses. In this thesis we have studied evolutionary responses to climate change in the perennial grass *Festuca ovina* using a long-term climate manipulation experiment at the Buxton Climate Change Impacts Laboratory (BCCIL), Derbyshire, UK. At BCCIL, natural grassland has been subjected to climate treatments since 1994. Previous studies of this system have found phenotypic and genetic differentiation between plants from the drought and control treatments. We do not know whether these responses represent evolution *sensu stricto*, if they increase plant fitness under new conditions, or whether evolved phenotypes alter the interactions of *F. ovina* with co-existing species. We addressed these knowledge gaps using methods including common environment experiments, a simulated drought experiment and quantitative genetic analyses. We have shown that there is heritable genetic variation in drought-relevant traits in *F. ovina*, and that traits, including germination timing and tiller growth rate, have evolved in response to climate change. Our results suggest that these responses do not increase fitness under a short-term drought. However, an increase in the abundance of *F. ovina* in the drought treatment at BCCIL suggests that its ability to persist, relative to the other species, is not diminished, challenging our concept of fitness. We have also found that evolutionary responses to climate change may result in species becoming less competitive, which will alter interactions between species. Our results demonstrate that evolutionary responses may provide populations with a mechanism to persist through climate change, but that evolutionary responses can be constrained by many processes, including the feedback from biotic interactions. Therefore, integrated studies, incorporating both ecological and evolutionary processes, are essential in order to better understand and predict the responses of plant populations and communities to climate change.

"Sing to the Lord with grateful praise;

make music to our God on the harp.

He covers the sky with clouds;

he supplies the earth with rain

and makes grass grow on the hills."

Psalm 147: 7-8

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1 Chapter 1. General introduction

1.1 Responses of plant communities to climate change

Biodiversity, the variability within species, among species and within ecosystems, faces a major threat from anthropogenic climate change (Bellard *et al.* 2012; Carey 2013). Global temperatures have increased by 0.72°C since 1951, and are predicted to increase by a further 0.3 – 0.7°C by 2035, accompanied by altered patterns of precipitation (Hartmann *et al.* 2013; Kirtman *et al.* 2013). Climate change is contributing to widespread changes to species distribution and interactions, and, ultimately, the reorganisation of communities (Parmesan 2007; le Roux & McGeoch 2008; Erschbamer *et al.* 2009; Gottfried *et al.* 2012; Moradi *et al.* 2012; Gornish & Tylianakis 2013; CaraDonna, Iler & Inouye 2014). Changes to plant community composition have been documented across a variety of habitat types, particularly characterised by an increase in the presence of warm-adapted species, a process termed *thermophilisation*[1] (Feeley *et al.* 2011; Gottfried *et al.* 2012; De Frenne *et al.* 2013). Changes to community composition, as a result of climate change, have also driven changes to ecosystem function and productivity (Hudson & Henry 2009; Traill *et al.* 2010; Gauthier *et al.* 2013).

One of the difficulties with observational studies of community and ecosystem responses to climate change is determining the specific cause of change (Magurran *et al.* 2010). In contrast, mesocosm experiments, in which the climate experienced by natural communities is manipulated directly, have proved

[1] Words in italics are defined in the glossary (Appendix 1).

particularly powerful in identifying causal impacts of the climate on biodiversity (Stewart *et al.* 2013). These experiments also allow us to mimic the climatic conditions expected in the future, to see whether natural communities will be able to withstand climate change. In general, studies that have manipulated temperature or precipitation have shown that biomass production and ecosystem processes, such as rates of photosynthesis, will increase under warmer and wetter conditions, but decrease under reduced precipitation (Wu *et al.* 2011; Unger & Jongen 2014). However, few manipulation studies are continued for extended periods of time, e.g. greater than 5 years (see table 1 in Unger & Jongen 2014), which may limit their utility for realistic predictions of the responses of communities to climate change over the long-term. For example, in a study applying a drought to steppe grassland annually for 11 years, it was found that drought plots had a significant reduction in total plant cover and altered community composition, but these effects were only evident after 4 and 7 years of drought respectively (Evans *et al.* 2011). This suggests that short-term experimental studies may miss the threshold at which community responses become apparent following a period of *resistance* to climate change (Hughes & Stachowicz 2004; Reusch *et al.* 2005). Focusing specifically on studies simulating drought conditions annually for more than 5 years, a variety of responses are evident. These include a reduction in species richness and changed community composition (Peñuelas *et al.* 2007; Prieto *et al.* 2009; Evans *et al.* 2011; Báez *et al.* 2013; Churchill *et al.* 2015), altered seedling establishment (Lloret *et al.* 2009) and changes to ecosystem functioning (Bates *et al.* 2006; Peñuelas *et al.* 2007; Fay *et al.* 2011; Evans & Burke 2013). However, other plant communities show little or no response to longer-term

drought treatments (Grime *et al.* 2008; Tielbörger *et al.* 2014). We still have limited understanding of the mechanisms underpinning the resistance and adaptation that we observe in natural communities to climate change.

Multiple processes are responsible for the changes we observe in natural communities in response to climate change; however, studies typically focus only on individual component processes. A key challenge for researchers is to understand how these component processes combine and interact, in order to predict the effects of climate change on natural communities. In the remainder of the chapter, we will first review the processes involved in plant responses to climate change at the level of populations, placing particular emphasis on adaptive evolutionary responses to climate change - a particularly understudied area. We then discuss how evolutionary and ecological processes integrate to result in responses at the level of communities. Finally, we introduce our study system, a long-term climate manipulation experiment, which specifically investigates plant responses to summer drought: such weather events are expected to increase in frequency in the UK (Jenkins *et al.* 2009).

1.2 Population level responses to climate change

1.2.1 Range shifts in response to climate change

The geographical ranges of plant species have shifted under warming temperatures during the last century (Parmesan & Yohe 2003; Chen *et al.* 2011). These changes in distribution are usually towards the poles and to higher altitudes,

as expected if species are tracking thermal optima (Parmesan & Yohe 2003; Root *et al.* 2003; Parmesan 2007; Chen *et al.* 2011). Species range shifts have been detected in many different ecosystems (Beckage *et al.* 2008; Kelly & Goulden 2008; le Roux & McGeoch 2008; Lenoir *et al.* 2008; Parolo & Rossi 2008; Feeley *et al.* 2011; Jump, Huang & Chou 2012; Greenwood *et al.* 2014). However, there is considerable variation in the strength of the distributional changes, with some studies finding little response to warming (Van Bogaert *et al.* 2011; Zhu, Woodall & Clark 2012), or even range shifts towards the equator, or downhill, counter to expectation (Crimmins *et al.* 2011).The processes driving shifts in species' geographical ranges differ between the trailing and leading range edges. In the former, losses are driven by mortality and reproductive failure, while in the latter, gains are driven by dispersal, colonisation and establishment processes (Jump, Mátyás & Peñuelas 2009; Corlett & Westcott 2013). The rates at which these processes operate depend on the species of interest, as well as local microclimate conditions, and any heterogeneity may result in unequal rates of range shift at the leading and trailing edges of a species (Jump, Mátyás & Peñuelas 2009). Furthermore, species may be tracking other key climatic variables that do not change consistently with temperature. For example, in some habitats, plant distributions may be constrained more tightly by moisture availability than by temperature *per se* (Crimmins *et al.* 2011).

1.2.2 Phenological responses to climate change

Climate warming induces an effective shift in the seasons, e.g. by advancing critical temperature thresholds for spring growth. In response, plants can adjust the timing of key lifecycle events such as budburst, growth and flowering time. These changes in phenology are among the best characterised responses to climate change (Parmesan & Hanley 2015). Numerous reviews and meta-analyses find consistent global patterns of earlier spring flowering and leaf emergence, in line with warming temperatures (Fitter & Fitter 2002; Parmesan & Yohe 2003; Root *et al.* 2003; Menzel *et al.* 2006; Thackery *et al.* 2010; Jeong *et al.* 2011). Summer and autumn events have also been observed to shift in line with warming temperatures, including an advance in the onset of fruit ripening and a delay in leaf senescence (Menzel *et al.* 2006). For some species the responses to warming are complex, and phenology is determined by the combined effects of winter warming (affecting vernalisation and dormancy cues) and spring warming (Cook, Wolkovich & Parmesan 2012). Furthermore, changes in phenology induced by warming can also be modified by changes in precipitation patterns (Jentsch *et al.* 2009; Hänel & Tielbörger 2015).

1.2.3 Evolutionary responses to climate change

Evolutionary responses provide one mechanism to facilitate the persistence of species through climate change (Pauls *et al.* 2013; Franks, Weber & Aitken 2014). Phenotypic change in response to climate change may be the result of either *phenotypic plasticity* or genetic change (Hansen *et al.* 2012; Merilä & Hendry 2014).

Demonstrating that phenotypic responses to climate change are evolutionary, as opposed to plastic, requires evidence that genetic change has taken place as a result of selection (Hansen *et al.* 2012; Merilä & Hendry 2014). Specifically, it should be demonstrated that there is heritable genetic variation in the trait of interest, that the trait is under climatic selection, and that there is a difference in the value of the trait as a result of changes in climate (Franks & Hoffman 2012; Merilä & Hendry 2014). Established methods for demonstrating evolutionary responses include common environment experiments, reciprocal transplant experiments, quantitative genetic studies, experimental evolution and space-for-time substitutions (see Table 1 in Merilä & Hendry 2014). Obtaining strong evidence for climate-driven evolution is difficult because it is hard to verify the specific environmental cause of genetic change (Merilä & Hendry 2014). Typically, demonstration of evolution in response to climatic selection requires multiple independent lines of evidence, through a combination of genetic analyses and field experiments (Anderson, Willis & Mitchell-Olds 2011; Merilä & Hendry 2014).

The capacity for evolutionary responses depends upon the presence of pre-existing genetic variation in phenotypes adaptive under climate change (Bradshaw & McNeilly 1991; Davis, Shaw & Etterson 2005; Jump & Peñuelas 2005). Rapid evolutionary responses to climate change have been documented in a variety of plants (Franks, Weber & Aitken 2014), including *Betula pubescens, Betula pendula* (Billington & Pelham 1991), *Brassica rapa* (Franks, Sim & Weis 2007) and *Thymus vulgaris* (Thompson *et al.* 2013). However, evolutionary change may not necessarily be adaptive, but may instead be maladaptive or neutral (Merilä & Hendry 2014). A further level of evidence is required to demonstrate that an adaptive evolutionary

response has occurred: specifically, that the evolved phenotype has a fitness benefit in the new environment (Franks, Weber & Aitken 2014). Note that this definition does not assume that the pace of adaptive evolution will be sufficient to keep up with environmental change. The strength or pace of evolution may be limited by genetic trade-offs among traits advantageous under climate change and other adaptive traits, or by low heritability in traits that are climatically adaptive (such as bud burst timing; Billington & Pelham 1991; Jump & Peñuelas 2005). Adaptive evolution that is sufficient to keep pace with climate change has been documented in only a few studies (Gonzalo-Turpin & Hazard 2009; Avolio, Beaulieu & Smith 2013; Sultan *et al.* 2013). Thus, our understanding of the capacity for adaptive evolution to enable plant populations to persist through climate change remains limited.

1.2.4 Evolution of drought adaptive traits

Water is essential for plant functioning. Drought is defined as a period when water availability is insufficient to meet the plant's requirements, resulting in water deficit, and is a key environmental challenge for plants (Taiz & Zeiger 2010). Global climate change models for North-West Europe predict that summer droughts are likely to increase in frequency and intensity (Jenkins *et al.* 2009). The sessile nature of plants requires that they adapt to drought *in situ*. This necessity has led to the evolution of a huge range of drought adaptations throughout the evolutionary history of plants, including changes to morphology and physiology, and molecular adaptations. Plants use two key adaptive strategies to maintain fitness under

drought. *Drought escape* is the ability of a plant to set seed and complete its lifecycle before the onset of drought. *Drought tolerance* is the ability of a plant to tolerate and survive low water availability in the established phase by adjustment of morphology and physiology (Farooq *et al.* 2012). Crop species have been particularly well studied with respect to drought, due to the damaging impact on food production. In these species, it has been shown that heritable genetic variation is present within many different traits associated with drought tolerance (Juenger 2013). However, the underlying genetic architecture may still impose constraints on the direction of evolution (Etterson & Shaw 2001; Etterson 2004b; Jump & Peñuelas 2005). One such example was found in the model species *Arabidopsis thaliana*, where Water Use Efficiency (WUE) is strongly positively correlated with flowering time; plants that flower earlier (an adaptation for drought escape) have low WUE (a drought tolerance trait) and vice versa (McKay, Richards & Mitchell-Olds 2003). This genetic architecture implies that selection cannot lead to the evolution of plants that have both high drought tolerance and drought escape abilities.

Studies of *local adaptation*, the relative fitness benefit of a resident individual in the environment to which it is adapted, relative to a non-resident individual, can help us to understand how plants adapt to drought in natural populations (Kawecki & Ebert 2004; Leimu & Fischer 2008). Reciprocal transplant experiments, comparing populations along clines of water availability, can inform us about patterns of selection on climatically-adaptive traits and the capacity of populations to adapt to water deficit (Etterson 2004b; Ramírez-Valiente *et al.* 2009; Kim & Donohue 2013). Climatic selection may drive evolutionary change towards

life-history strategies that are more adaptive under drought. Such studies have provided examples of trait differentiation towards both drought escape and drought-tolerant life-history strategies (Etterson 2004a; Petrů *et al.* 2006; Ramírez-Valiente *et al.* 2009; Brouillette *et al.* 2013).

1.3 Integrating responses of communities to climate change

1.3.1 Climate driven evolution altering communities

Adaptive genetic variation has an important role in structuring ecological communities (Fritz & Price 1988; Johnson, Lajeunesse & Agrawal 2006; Lankau & Strauss 2007; Jung *et al.* 2010) and ecosystem dynamics (Hughes & Stachowicz 2004; Schweitzer *et al.* 2004; Reusch *et al.* 2005; Schöb *et al.* 2015). Studies in which the levels of genetic diversity or species richness of a community have been controlled show that both the community structure and species diversity of the community can change depending upon the levels of genetic diversity of the constituent species (Booth & Grime 2003). These studies show that both *direct genetic effects* and *interspecific indirect genetic effects* are important for regulating plant community structure (Whitlock *et al.* 2011). With respect to studies on climate change, research into the responses of plants has tended to focus on individual species, without taking into account the context of an ecological community of coexisting species (Gilman *et al.* 2010; Walther 2010). However, this ecological context can drive evolutionary change and, equally, evolution can alter community structure and ecosystem dynamics (Haloin & Strauss 2008; Urban *et al.*

2012). Therefore, in order to predict more accurately the responses of plant communities to climate change, research must integrate both ecological and evolutionary processes.

The combined ecological and evolutionary aspects of plant responses to climate change can interact with each other in unexpected ways. Biotic interactions with co-existing species can constrain or facilitate adaptation to the climate; the importance of such biotic interactions can vary depending on the level of abiotic stress (Bischoff *et al.* 2006; Brooker 2006; Liancourt & Tielbörger 2009; Ariza & Tielbörger 2011). Alternatively, evolutionary responses to climate change can alter biotic interactions. For example, research on *Brassica rapa* demonstrated rapid adaptive evolutionary responses to drought through the evolution of earlier flowering time (drought escape; Franks, Sim & Weis 2007; Franks & Weis 2008). However, subsequent work has shown that this was accompanied by increased susceptibility to a fungal disease, suggesting that advantageous adaptation in one trait may trade-off with other important adaptive traits (O'Hara, Rest & Franks 2015). This illustrates how the interplay between ecological and evolutionary mechanisms is key to a fuller understanding of the responses of communities to climate change.

Genetic diversity may also be important in enhancing the *resistance* and *resilience* of a community to environmental change (Hughes & Stachowicz 2004; Reusch *et al.* 2005). Specifically, intraspecific genetic diversity may provide a mechanism, via complementarity or by direct genetic effects of well-adapted genotypes (the selection effect; Loreau & Hector 2001), to stabilise communities against the effects of perturbation, such as an extreme climatic event (Hughes &

Stachowicz 2004; Hughes & Stachowicz 2011). In their work on *Zostera marina*, Reusch *et al.* (2005) manipulated populations of *Z. marina* to contain different numbers of genotypes. They found that communities with greater genetic diversity had greater resilience to a natural extreme heat event. Other examples of the role of genetic diversity in the response of a community to disturbance include studies of species invasion (Vellend, Drummond & Tomimatsu 2010), disturbance from grazing (Hughes & Stachowicz 2004), and drought events (Jung *et al.* 2014). These examples demonstrate the importance of intraspecific genetic diversity for providing a potential mechanism by which populations and communities may resist environmental change.

1.4 Study system

1.4.1 Buxton Climate Change Impacts Laboratory (BCCIL)

Long-term mesocosm experiments, in which the climate experienced by natural communities is manipulated directly, have proved particularly helpful for understanding the impacts of climatic selection on biodiversity and the processes underpinning these responses (Stewart *et al.* 2013). At the Buxton Climate Change Impacts Laboratory (BCCIL), Derbyshire, 3 m × 3 m plots of calcareous, species-rich grassland have been subjected to experimental climate change since 1994 (Grime *et al.* 2000; Grime *et al.* 2008). Treatments at this site include winter warming, supplementary summer rainfall and summer drought, along with factorial combinations of these and non-manipulated control plots (Grime *et al.* 2000). Each

climate environment is replicated in five plots in a randomised block design. Winter warming is imposed from November to April each year through heating cables fastened to the soil surface, which raise soil surface temperature to 3°C above the ambient temperature. Supplementary summer rainfall is applied from June to September by the addition of deionized water equivalent to a 20% increase over the average precipitation in the preceding 10-year period. Summer drought treatment is applied during July and August using automatic rain shelters, which move over the plots when it starts raining and move back to an off-plot position when the rain stops (Figure 1.1). This results in a significant reduction in soil surface water potential by the end of the two-month treatment (-1100 kPa in drought plots vs. -20 kPa in controls plots at 5 cm depth; see Figure 4 in Fridley *et al.* 2011).

Variation in soil depth provides the greatest natural source of environmental heterogeneity within grassland plots, ranging from 0 cm (bedrock) to greater than 40 cm in depth. Substantial changes in soil depth (> 20 cm) frequently occur in short horizontal displacements (< 10 cm), creating a fine-scale spatial mosaic in soil depth heterogeneity.

Previous studies at BCCIL have found that communities within the plots have shown resistance to the simulated climate change, changing little in community structure (Grime *et al.* 2000; Grime *et al.* 2008). However, at fine spatial scales there has been significant reorganisation of community structure, strongly influenced by microclimatic conditions, particularly soil heterogeneity (Fridley *et al.* 2011; Fridley *et al.* 2016). High levels of genetic and species diversity, the fine-scale habitat heterogeneity, and local adaptation are all thought to be important components of the resistance to climate change demonstrated by this community

(Grime *et al.* 2008; Fridley *et al.* 2011; Ravenscroft, Fridley & Grime 2014; Ravenscroft, Whitlock & Fridley 2015; Fridley *et al.* 2016).

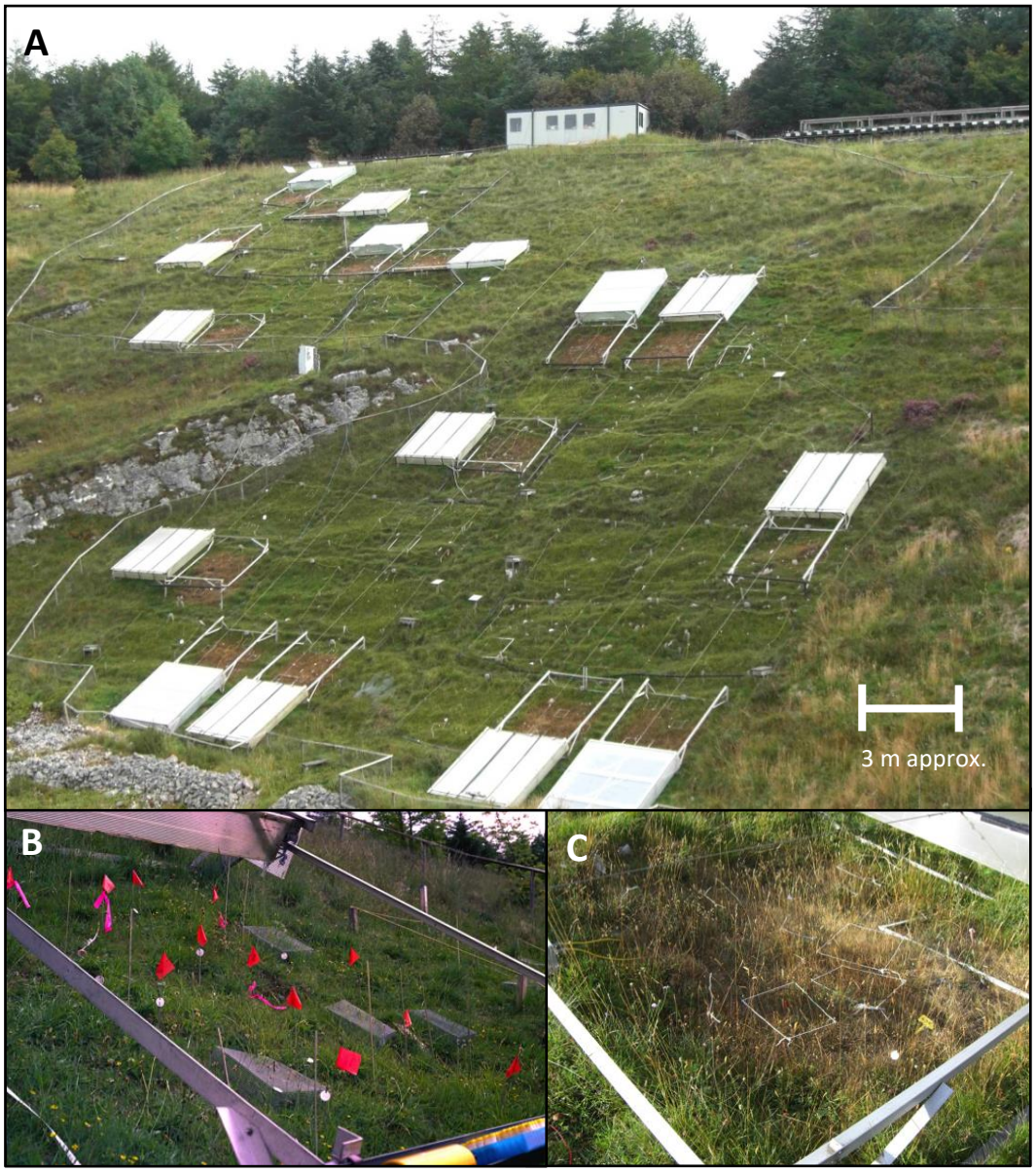

Figure 1.1 A) Experimental plots at Buxton Climate Change Impacts Laboratory, Derbyshire. Rain shelters are used in the summer drought treatments to prevent rain falling on plots during the months of July and August. Plots are 3 m × 3 m. Close-up images of experimental plots at BCCIL; **B)** Control plot **C)** Drought-treated plot. Vegetation damage as a result of drought can be seen in the drought-treated plot. Photograph credit: A & C: Andrew Askew, B: Raj Whitlock, 2010.

On-going research on the BCCIL community suggests that some species are showing evolutionary responses to selection (Whitlock, unpublished data). In one study, the *parent microcosm experiment*, clonal replicates of four species, *Festuca ovina* L., *Koleria macrantha* (Ledeb.) Schult.*, Carex panicea* L. and *Lotus corniculatus* L. , collected from the drought and control plots at BCCIL, have been grown in a common environment, and their phenotypes measured. It has been found that traits of *F. ovina*, *L. corniculatus* and *C. panicea* collected from the drought plots at BCCIL have diverged phenotypically from the control population (Whitlock, unpublished data). In another study, on *Plantago lanceolata* L. also collected from BCCIL, it has again been shown that there is phenotypic differentiation between plants from the drought plots compared to those from the control (Ravenscroft, Fridley & Grime 2014). These results demonstrate heritability in the broad sense in traits relevant to drought-adaptive strategies (Lynch & Walsh 1998), and suggest evolutionary responses to climatic selection are occurring in some of the species at BCCIL. However, we do not know whether these responses are evolution *sensu stricto* or carry-over effects from the field. In another study, using outlier analyses of amplified fragment length polymorphism marker genotypes, little evidence was found to support a genomic signature of climatic selection that is consistent with an adaptive evolutionary response (Ravenscroft, Whitlock & Fridley 2015). There is still inconclusive evidence on whether adaptive evolution is occurring at BCCIL in response to climatic selection.

The BCCIL system provides a unique study with which to examine evolutionary responses to long-term climatic selection in an intact ecosystem and to assess the potential ecological impacts of evolutionary responses to climate

change. At BCCIL the drought treatment has resulted in the greatest changes, driving changes in species abundance, diversity and biomass productivity (Grime *et al.* 2008; Fridley *et al.* 2011). Consequently, in this study we focus on evolutionary responses to the drought treatment in comparison to the control treatment.

1.4.2 Study species

Festuca ovina L. (sheep's fescue) is a long-lived, perennial grass, producing dense tussocks (Stace 2010). Flowers are hermaphrodite, wind pollinated and self-incompatible (Watson 1958). It is found as a diploid (2*n* = 14), tetraploid (2*n* = 28) and hexaploid (2*n* = 42) across its range (Watson 1958; Sampoux & Huyghe 2009). It is widespread in both Britain and Ireland and elsewhere in Europe (Preston, Pearman & Dines 2002), and is a defining species of many calcareous, species-rich grasslands (for example those grasslands comprising the National Vegetation Classification (NVC) community *Festuca ovina – Avenula pratensis* CG2 (Rodwell 1992)).

Festuca ovina is the most abundant species in the BCCIL grassland community and has increased in abundance under simulated drought at BCCIL (Fridley *et al.* 2011). Results from the *parent microcosm experiment,* described above, have found that individuals of *F. ovina* from the drought plots at BCCIL have an earlier flowering time, less reproductive effort and a smaller specific leaf area, relative to individuals from control plots, when grown in a common environment. Thus, the response of this species to simulated climate change at BCCIL has the potential to affect many other co-existing species.

1.4.3 This thesis

In this thesis we examine evolutionary responses to long-term climatic selection at BCCIL in *F. ovina*. The research described in this thesis builds on the work carried out at BCCIL using *F. ovina* as a study species, and focuses solely on differences in the responses between plants from the drought and control treatments. In Chapter 2 we describe the construction of an F1 progeny array from *F. ovina* parent plants collected from drought-treated and control plots at BCCIL. We develop and use microsatellite markers to create a pedigree for the F1 progeny plants via paternity analysis. The F1 progeny array and associated pedigree are a fundamental experimental resource underpinning the other experiments described in this thesis. We use the pedigree (i) to assess aspects of the *F. ovina* mating system in relation to ancestral climatic conditions (including male reproductive success and mating constraints) and (ii) to assess evolutionary differentiation in germination timing and probability.

In Chapter 3 we use a common environment experiment to determine the heritability and genetic architecture underpinning key plant traits that are potentially adaptive under drought. We use these data to identify possible genetic constraints on climate-driven evolutionary responses in *F. ovina*.

In Chapter 4 we measure ploidy and genome size in the *F. ovina* collected from BCCIL and ask whether these have altered under long-term climatic selection. Using these data, we then investigate whether intraspecific variation in genome size correlates with key plant phenotypes that are potentially adaptive under drought.

In Chapter 5 we use a simulated drought treatment applied to a microcosm experiment, growing *F. ovina* along with a community of coexisting species, in order to study the integration of evolutionary and ecological processes and to assess their consequences. Namely, we determine whether evolutionary responses are expressed in the presence of coexisting species, whether evolutionary responses are adaptive under a simulated drought, and whether evolutionary responses have consequences for coexisting species.

Finally, in Chapter 6, we discuss the results of this thesis in the broader research context. We consider to what extent we find consistent patterns of adaptive evolutionary responses to climate change and whether genetic variation in life-history strategy will enable *F. ovina* to persist through a changing climate.

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2 Chapter 2. Climate-driven evolutionary change in reproductive and early-acting life-history traits in the grass *Festuca ovina*

2.1 Abstract

Reproductive and early-acting life-history traits such as seed mass and germination timing may be particularly important determinants of fitness under a changing climate, since they strongly influence demography and survival in the next generation of plants. However, evolutionary changes in these traits are likely to be cryptic in many natural populations, going unobserved. It is important that we understand the evolution of these traits, so that we can determine their contribution to adaptation and population persistence under a changing climate. In this chapter, we describe the establishment of an F1 progeny array in the grass *Festuca ovina*. We then use this resource to examine climate-driven evolutionary change in reproductive output, and in a key early-acting life-history trait, germination timing. We collected established plants of *F. ovina* from the Buxton Climate Change Impacts Laboratory (BCCIL), where natural grassland has been subjected to experimental climate change for 17 years. Plants were collected from drought-treated and control plots, and following establishment in a common garden, were used to create a sexual F1 progeny array by open pollination. Microsatellite markers were developed and used to reconstruct the bi-parental pedigree of the F1 *F. ovina* progeny. We measured seed mass and the timing of germination. F1 seed with ancestry in drought-treated plots at BCCIL germinated

significantly later than seed derived from individuals from control plots. We also found that male reproductive success in plants collected from drought plots was lower than those from control plots. Furthermore, our pedigree revealed that mating among parents of the F1 progeny was assortative with respect to flowering time, a trait that has been altered by the simulated drought treatment at BCCIL. Our results are consistent with climate-driven evolutionary change in male reproductive success and germination latency in *F. ovina*. These results emphasise the importance of early-acting life-history traits for the adaptive responses of plants to climate change, and demonstrate how adaptive responses may be reinforced by reproductive isolation.

2.2 Introduction

Recent studies are showing how plant populations are evolving in response to climate change, and which traits respond (Etterson & Shaw 2001; Etterson 2004a; Etterson 2004b; Gonzalo-Turpin & Hazard 2009; Franks, Weber & Aitken 2014). If such evolution is adaptive, then it may provide an important buffering mechanism for natural populations, allowing them to persist through climate change (Hoffmann & Sgro 2011). Plant reproductive success is typically measured via seed output. However, this measure only accounts for the maternal component of reproductive success. The reproductive fitness of hermaphrodite plants is comprised of both female and male components. In this context, male reproductive success is the number of offspring a plant sires by pollen (Primack & Kang 1989) and

is very difficult to quantify in natural populations (Bertin 1988). Consequently, comparatively few studies have investigated the factors that influence it, and most of these have examined the role of biotic factors, such as pollination, in shaping patterns of male reproductive success (Schaeffer, Manson & Irwin 2013). Our understanding of the effects of abiotic variables on male fitness is limited, and the impact of climate change on male reproductive success has been almost entirely overlooked (but see Marshall *et al.* 2010). A few studies have, however, demonstrated that male fitness in plants can be affected by temperature (Jóhannsson & Stephenson 1998; Pasonen, Pulkkinen & Kärkkäinen 2002), CO_2 concentration (Marshall *et al.* 2010) and nutrient availability (Young & Stanton 1990; Lau & Stephenson 1993; Lau & Stephenson 1994; Poulton, Koide & Stephenson 2001). More research is needed to understand the evolution of male reproductive success under climate change and the resulting effects on total plant fitness.

Since male reproductive success is difficult to measure in natural populations, it is often assumed to correlate linearly with female fitness, and therefore, to contribute equally to total fitness: an assumption that is frequently invalid (Ennos & Dodson 1987; Devlin & Ellstrand 1990; Conner *et al.* 1996). A number of studies have used proxies for male reproductive success; however, the assumption that these measures correlate closely with the actual number of offspring sired does not always hold (Melser, Rademaker & Klinkhamer 1997; Marshall, Shaner & Oliva 2007). Reliable estimates of male reproductive success require a direct measure of the number of offspring sired i.e. measurement of paternity (Snow & Lewis 1993). In some systems, paternity can be determined using

phenotypic markers such as flower colour (Young & Stanton 1990; Lau & Stephenson 1993). Alternatively, researchers can employ parentage analysis using DNA markers, which allows the reconstruction of the pedigree (Snow & Lewis 1993). Studies directly measuring male reproductive success (the number of offspring sired) have shown that individual plants vary in their functional gender (their relative success through male and female reproduction; Snow & Lewis 1993). Furthermore, these studies demonstrate that the proportion of investment in male or female output can vary from year to year (Ennos & Dodson 1987; Broyles & Wyatt 1990; Devlin & Ellstrand 1990; Conner *et al.* 1996). The impact of climate change on the evolution of male or female reproductive strategies, and resulting shifts in functional gender, is unknown. However, the evolution of mating systems may have fundamental impacts on local demography, altering the probability of population persistence (Etterson & Mazer 2016). It may also be critical in determining patterns of gene-flow between populations, and the maintenance of genetic variation within populations (Barrett 2003).

Germination is initiated in response to a species-specific combination of environmental cues including levels of soil moisture, seasonal temperature thresholds, and changes in day length (Grime *et al.* 1981; Fenner & Thompson 2005). The environmental conditions in which a seed germinates are those under which the establishing plant must grow. Thus, the correct timing of germination is crucial for survival, and is under strong selection (Donohue *et al.* 2010; Walck *et al.* 2011). Climatic factors, such as temperature and precipitation are key determinants of germination timing and success. It is likely, therefore, that the alteration of temperature and precipitation regimes during climate change will interfere with,

and modify, existing cues for germination (Walck *et al.* 2011). Furthermore, germination and early establishment traits have significant heritable variation and are under strong natural selection, often contributing strongly to *local adaptation*[2] (reviewed by Donohue *et al.* 2010; and Baskin & Baskin 2014). Thus, we can expect these traits to evolve during climate change (Donohue *et al.* 2010).

The timing of germination with respect to season and environmental conditions forms a key part of a plant's adaptive life-history strategy, defining habitat preference and affecting community structure (Fenner & Thompson 2005; Donohue *et al.* 2010; Baskin & Baskin 2014). For example, Schütz (2000) found that most temperate *Carex* species are able to germinate at 25°C, but only species from woodland habitats were also able to germinate at 10°C. This allowed the woodland species to germinate early and establish before the tree canopy closed, securing a narrow establishment niche. Changes to the timing of seed germination also influence population dynamics, and thus may have knock-on effects for community composition (Walck *et al.* 2011; Parmesan & Hanley 2015; Jiménez-Alfaro *et al.* 2016).

To study the evolution of reproductive and early-acting life-history traits in response to climate change, we have used the Buxton Climate Change Impacts Laboratory (BCCIL) study system. At BCCIL a natural grassland has been subjected to a range of experimental climate change treatments since 1994. Each climate treatment is replicated in five plots in a randomised block design. The grassland

[2] Words in italics are defined in the glossary (Appendix 1)

community at BCCIL has shown *resistance* to simulated climate change, with community composition remaining relatively constant (Grime *et al.* 2000; Grime *et al.* 2008). However, at fine scales there has been significant reorganisation of species abundance. We chose to focus this investigation on the drought treatment at BCCIL because, amongst the treatments imposed at BCCIL, the drought treatment has driven the greatest changes in species abundance, and has also altered biomass productivity (Fridley *et al.* 2011). *Festuca ovina,* the focal study species of this thesis, has increased in abundance in the drought plots. *Festuca ovina* is a perennial grass, which is wind pollinated, with hermaphroditic, self-incompatible flowers (Stace 2010). Our preliminary research on phenotypic responses to drought treatment in this species has indicated significant broad-sense genetic change in flowering time, reproductive effort and specific leaf area between drought-treated and control plots (R. Whitlock, unpublished data). DNA marker analyses also indicate significant genetic change among climate treatments in *F. ovina* (Ravenscroft, Whitlock & Fridley 2015). However, we do not know whether these responses are evolution *sensu stricto,* or carry-over effects from the field. Also, we do not know how evolution may be occurring in early-acting traits.

In this chapter, we use *F.ovina* individuals collected from a long-term climate manipulation experiment at the Buxton Climate Change Impacts Laboratory (BCCIL) to determine whether climate change drives evolutionary changes in reproductive and germination traits. We collected *F. ovina* plants from drought and control plots at BCCIL, and subsequently developed microsatellite markers for this species. We used the markers to establish an F1 progeny array from seed derived from open pollination of the field-collected plants, via pedigree reconstruction. The

F1 plant material and associated pedigree represent central resources for the study of climate-driven evolutionary responses in *F. ovina*, used in this and future chapters. Finally, we used the F1 progeny array to investigate evolutionary responses to drought in two key components of plant fitness: reproductive and germination traits. We find evidence for climate-driven evolutionary change in the timing of seed germination, in male reproductive success and for assortative mating by climate treatment and flowering time.

2.3 Methods

2.3.1 Study site and species

This work is based on plant material collected from BCCIL (specifically, drought-treated and control plots), where climate treatments have been applied to intact 3 m $\times$ 3 m plots of calcareous grassland ecosystem for 17 years (see Chapter 1, Section 1.4 for further details on BCCIL). In brief, the drought treatment has been imposed for two months, through July and August, annually since 1994 (Grime *et al.* 2008). During the drought treatment, rain shelters move over the grassland plots during precipitation, to intercept rainfall, subsequently returning to an off-plot position. This treatment results in a significant reduction in surface soil water potential by the end of the two-month drought treatment: -1100 kPa in drought plots compared to -20 kPa in controls (Fridley *et al.* 2011).

2.3.2 Collection and propagation of F. ovina clonal lines from BCCIL

In July 2010, *F. ovina* individuals were collected from drought and control plots at BCCIL by R. Whitlock, after 17 years of climate manipulation. Thirty individuals were collected from each of these climate environments (drought and control; six individuals per plot, per treatment). A stratified random sampling design was used to collect the plants, which were immediately potted in cell trays in John Innes No. 1 compost (further details provided in Appendix 2, Section A2.1). Small bunches of 4–8 connected tillers were recovered from each sampled plant in the field. Soil depth was recorded at every sampling point.

Upon return from the field (July 2010), the plants were allowed to establish in 3 L pots containing a 3:1 mix of John Innes No. 1 potting compost and medium grade Perlite (LBS Horticulture), at the Arthur Willis Environment Centre (AWEC, University of Sheffield, UK). The plants were watered daily with mains water during the month following their collection from BCCIL. After this they were watered once or twice a week to maintain soil moisture. Plants were kept at AWEC over winter until April 2011.

On 19 April 2011 the plants (hereafter clonal lines) were moved to Ness Botanic Gardens, University of Liverpool, UK, and housed there in purpose-built raised "bays" (Figure 2.1). They were maintained by biomass clipping above 25 mm every September to mimic grazing and to promote clonal growth, and by seed head removal during summer 2011, to prevent self-seeding. The clonal lines received natural rainfall, which was supplemented with "borehole" water (a pumped ground-water supply) during dry periods. This set of 59 clonal lines (one individual died following collection) is referred to, hereafter, as the *parent clonal library*.

Figure 2.1 The purpose-built experimental bays at Ness Botanic Garden, University of Liverpool, UK where the parent clonal library and offspring clonal library were housed and maintained. The bays are raised above the ground to prevent rabbit damage and are covered by netting to prevent damage by birds.

2.3.3 Creation of an F1 progeny array

By June 2012 the parent clonal library plants had formed large dense tussocks of approximately 100-200 tillers; we used this material to create an F1 progeny array. Our aim was to allow plants in the parent clonal library to mate via natural wind pollination, while minimising the spatial determination of mating arising from pot location. Each maternal parent can also act as a paternal parent (though not through self-pollination). The 59 parent clonal lines (collected from BCCIL in 2010) were growing in the separate 3 L pots in which they had established over winter. These were located outside in a single raised bay at Ness Gardens, arranged in a rectangular array of 4 rows of 15 pots. Each day (Monday to Friday), during flowering, pot locations were permuted by exchange of four pairs of

randomly-selected pots from outside rows to inside rows. Each pot was moved at least once every 8 working days.

During July 2012 (28/06/12–19/07/12), the number of flowering tillers on each of the plants in the parent clonal library was counted and their seed was collected. From these bulk collections of seeds, sixteen seeds from each parent clone were selected at random and weighed individually (i.e. a balanced design with respect to maternity). These 16 seeds were placed on filter paper (90 mm) within a 90 mm petri dish with 1.4 ml of Milli-Q ultra-high pure water (18.2 MΩ□cm). Seed dishes were placed in a fridge for 24 hours before being moved to an indoor space at room temperature with natural lighting. All the petri dishes were watered twice a week with 1 ml of Milli-Q ultra-high pure water. Each dish was checked daily and the germination date of each individual seed was recorded. Germination was scored as the emergence of the coleoptile (the protective sheath that encases the first emerging shoot). Recording of germination started on 07/08/2012 and the final observations were recorded on 04/10/2012. Seeds that had not germinated by this final date (after 59 days of observation) were recorded as having failed to germinate.

Eight seedlings were selected randomly from those that germinated, for each parent clonal line, and planted into seed trays (24 cell trays, each pot 5 cm × 5 cm × 5 cm) containing a 1:2:1 mix of natural rendzina soil, John Innes No. 1 compost and perlite. These 472 individuals (comprising the *offspring clonal library*) were subsequently allowed to establish outdoors in raised bays at Ness Botanic Gardens, and were managed as clonal lines, as described for the parent clonal library.

2.4 Genetic markers

2.4.1 Leaf tissue sampling

Fresh, green leaves (2–5) were dried for subsequent DNA extraction in 2 ml screw top tubes containing self-indicating silica gel (1–3 mm grain size, Merck KGaA, Germany). Leaf tissue samples were collected from each of the plants in the parent clonal library (n = 59) and the offspring clonal library (n = 457; 6 plants did not establish). A replicate collection of leaf tissue from 37 clonal lines (selected at random from the parent clonal library) was also taken to allow estimation of genotyping error.

2.4.2 Genomic DNA extraction

DNA was extracted from 2-5 dried leaves (approx. 4 – 10 mg) using a high-throughput micro-titre plate-based protocol modified from Whitlock *et al.* (2008), full details provided in Appendix 2, Section A2.2. DNA quality and quantity were assessed by gel electrophoresis (0.8% agarose run at 100 volts for 1 hour) with a size standard (Hyperladder 1, Bioline, UK) and with quantitative standards, Lambda DNA, 48502bp, (Thermoscientific, UK) at concentrations of 10 ng μl^{-1}, 20 ng μl^{-1} and 50 ng μl^{-1}. Extracted gDNA samples had a concentration in the range 10–20 ng μl^{-1}, and were between 20 kb and 50 kb in fragment length.

2.4.3 Microsatellite marker development

Microsatellite sequences were identified from transcriptome sequence data of *F. ovina* (i.e. from genic sequences). The transcriptome sequence data were created as part of a parallel genomic investigation that aimed to establish genomic resources for *F. ovina* in order to identify climatically-adaptive gene variants (R. Whitlock, unpublished). The transcriptome data had been obtained from a pooled sample of RNA extracted from leaf, stem, root, and inflorescence tissue of a single *F. ovina* individual from the Buxton population. This sample was sequenced on the Illumina HiSeq 1000 platform (2 × 100 bp reads, ¼ of a lane) and the resulting 98,507,346 reads were assembled using TRINITY (Grabherr *et al.* 2011). This generated 119,065 contigs, with a mean length of 854.8 bp and an N50 of 1,074 bp.

We used the software MSATCOMMANDER (Rozen & Skaletsky 2000; Faircloth 2008) to identify dinucleotide repeats motifs within the *F. ovina* transcriptome. MSATCOMMANDER was used to design primers automatically for flanking regions of loci with greater than 8 dinucleotide repeats, using Primer3 (Rozen & Skaletsky 2000). This resulted in the design of primers for 163 possible microsatellites, excluding possible duplicated sequences. BLAST searches (Altschul *et al.* 1990) were performed for each putative microsatellite locus using the blastn algorithm (optimising for somewhat similar sequences), with default parameters (expected threshold = 10, match/mismatch scores = 2,–3, gap costs = Existence 5, Extension 2). Five of the selected transcriptome sequences matched bacterial sequences with 100% identity and >90% coverage. These sequences were excluded from the dataset. Primers were ordered for a random sample of 48 of the remaining microsatellite loci, and were screened for amplification and polymorphism.

2.4.4 Primer screening and PCR

Primer screening was carried out using 5' end-labelled oligonucleotides (with 6-FAM fluorescent labels; Sigma-aldrich, UK). PCR for primer screening was carried out in a 3 µl volume within 384 well plates (ThermoFisher Scientific, UK). Each reaction contained 1.5 µl Qiagen Multiplex PCR Master Mix (Qiagen PCR Buffer, 6 mM $MgCl_2$, dNTP Mix and HotStartTaq DNA Polymerase), 0.3 µl of a primer mix containing each primer at 2 µM (final primer concentration, 0.2 µM), 0.3 µl of Q-Solution (Qiagen), 0.3 µl of RNase-Free Water and 0.6 µl of template DNA. Thermo-cycling conditions were as follows: initial denaturation at 95°C for 15 minutes; 35 cycles of 94°C for 30 seconds, 58°C for 90 seconds, 72°C for 60 seconds; and a final extension at 60°C for 30 minutes.

Microsatellite fragments were separated by length using capillary gel electrophoresis on an ABI Prism 3130 XL. Microsatellite allele fragment lengths were determined using an internal size standard (LIZ 500; ThermoFisher Scientific, UK). Nine primer pairs amplified consistently and showed high levels of polymorphism within a batch of test individuals, and these 9 primers were selected to design multiplex reactions for. Further details of primer screening methods are provided in Appendix 2, Section A2.3.

2.4.5 Genotyping

Two multiplex PCR reactions were designed for the 9 chosen microsatellite loci, using the Qiagen Multiplex kit, as set out above (6 primers were re-ordered with VIC or NED fluorescent dyes to enable the necessary spectral discrimination of

PCR products). Each multiplex PCR reaction was optimised by fine-tuning annealing temperature and cycle length (Table 2.1). This resulted in final cycling conditions for the two multiplexes as follows. *Festuca* Multiplex 1: initial denaturation at 95°C for 15 minutes; 35 cycles of 94°C for 30 seconds, 58°C for 90 seconds, 72°C for 60 seconds; and a final extension at 60°C for 30 minutes. *Festuca* Multiplex 2: initial denaturation at 95°C for 15 minutes; 32 cycles of 94°C for 30 seconds, 64°C for 90 seconds, 72°C for 60 seconds; and a final extension at 60°C for 30 minutes.

Table 2.1. Characteristics of the 9 polymorphic microsatellite markers developed for *F. ovina* and the multiplex reaction in which they were processed.

Primer ID	Forward primer sequence including florescent label (5'-3')	Repeat motif (5' – 3')	Allele size range	*N* alleles	Multiplex	M_t (°C)
T_02	F: (6-FAM)-TCCTCGATGAAGAACCCGTC R: TCCCATCTTCACCCATTTCTTC	AG	293–321	11	1	58
T_26	F: (NED)-TCGTCAAGATGGCAAACGATG R: GAATCGGCAGGAAAGGAACG	AT	363–373	6	1	58
T_28	F: (6-FAM)-AGTCCAGTCGCTGCAGATAG R: CCTGTCCTCTTGGCCGATAG	TG	221–262	16	1	58
T_35	F: (NED)-ACACGGTCACAGTCTCACAG R: TTCATTCCCTAGCTCCGTCG	TC	193–206	9	1	58
T_42	F: (VIC)-CCCAAGTTTAGACCGTTGCC R: TCAGGGAGCGGAATGTAGTG	CT	332–368	17	1	58
T_06	F: (6-FAM)-CTCTGATATGAGGCCGCAAC R: TGCAGCGTAACAGACTTTCAC	CT	179–235	19	2	64
T_19	F: (6-FAM)-TTGGAGGACAGTGGGACTTG R: CTTGTGATTCAGGGCGTTGG	CT	404–456	20	2	64
T_23	F: (NED)-TCTGGGACGAAGACAGCATC R: CGATGTTTAACAGGGTCAAGGG	AG	429–456	16	2	64
T_25	F: (6-FAM)-AGGGATTAGGACACGAGAACC R: TGACCTCCTCTGATCTGCAC	AG	129–140	10	2	64

M_t = annealing temperature of multiplex.

2.4.6 Microsatellite scoring

Microsatellite loci were scored using semi-automated genotyping methods set up for each multiplex within GeneMapper Version 3 (Applied Biosystems). We made up to four allele calls for each locus per individual, since our population of *F. ovina* is tetraploid (see Chapter 4). Further details of the method for scoring microsatellites can be found in Appendix 2, Section A2.4. All allele calls were manually checked for correct allele assignment and were corrected where required. Any samples where allele calling was ambiguous (because of poorly amplified peaks) were removed from the project. PCR and genotyping was repeated for these failed samples.

2.5 Statistical analysis

2.5.1 Genetic data analysis

Allele counts from microsatellite genotypes indicated that *F. ovina* from BCCIL are tetraploid (this was later confirmed by chromosome counting and flow cytometry, see Chapter 4). For this reason, genetic data were analysed in R (R Development Core Team 2008) using the package POLYSAT, which provides a framework for population genetic analysis for polyploid species (Clark & Jasieniuk 2011).

The *assignclones* function in POLYSAT (Clark & Jasieniuk 2011) was used to test whether individuals from the parent clonal library were unique clones. First, a genetic distance matrix was calculated using the Bruvo distance measure, and the

meandistance.matrix2 function, with selfing rate set to 0.1 (Clark & Jasieniuk 2011). Next, the *assignclones* function was applied to this matrix, with the threshold level (which indicates the maximum genetic distance between two individuals that will be placed in the same clonal group) set at 0.2, following the guidelines in Clark (2014).

We used the probability of identity, $P_{(ID)}$, between sibs, as a measure of the power of our microsatellite markers to discriminate among related plant individuals. $P_{(ID)}$ is defined as the probability that two individuals in a population will have the same genotype at multiple marker loci if selected at random (Waits, Luikart & Taberlet 2001). The microsatellite genotypes were converted to binary data, representing the presence and absence of individual microsatellite alleles, using the *genambig.to.genbinary* function in POLYSAT (Clark & Jasieniuk 2011), and then treated as dominant markers. $P_{(ID)}$ was calculated for this dataset (excluding replicated samples) following Waits, Luikart & Taberlet (2001) using the equation for dominant markers,

$$P_{(\mathrm{ID})\mathrm{sib}} = 1 - \{(3/2p)(q^2)\},$$

where p is the allele frequency and q is the null allele frequency, representing absence of the allele. Allele frequencies were calculated using Zhivotovsky (1999) method for dominant genetic data, using a non-uniform prior. $P_{(ID)}$ was calculated separately for each locus, and then chain multiplied across loci to calculate the multi-locus $P_{(ID)}$, summarising the total discriminatory power of our microsatellite markers.

2.5.2 Statistical analyses using Bayesian modelling

Many of the statistical analyses in this thesis are carried out using Bayesian models, in particular, to conduct parentage analysis and to analyse Generalised Linear Mixed-effects Models (GLMMs). We therefore provide a brief outline of Bayesian methods and terminology. Bayesian analysis is a statistical approach that applies Bayes' theorem, to combine prior knowledge and information from data, to provide an improved view on a particular hypothesis or question (Gelman *et al.* 2004; Korner-Nievergelt *et al.* 2015). Classical statistical analysis asks what is the probability of the data being observed given a certain hypothesis; Bayesian analysis differs conceptually, asking what is the probability that our hypothesis is true, given the data available (Ellison 2004). The *"prior distribution"* expresses the knowledge we already have about the data and parameter(s) of interest (Korner-Nievergelt *et al.* 2015). The "*posterior distribution"* describes what we know about the parameter(s) of interest once the data are observed in light of the model and our prior distribution (Korner-Nievergelt *et al.* 2015). A wide range of simple Bayesian problems can be solved analytically, but many other modelling problems, including some GLMMs, cannot. In these latter cases, Bayesian approaches often use Markov chain Monte Carlo simulations (MCMC) to construct a posterior distribution iteratively, using, for example, Metropolis-Hastings updates or slice sampling to sample parameter values. MCMC allows the model to explore multivariate parameter space and to converge upon a region with high likelihood, known as the stationary distribution (Gelman *et al.* 2004; Korner-Nievergelt *et al.* 2015). From independent samples of the stationary distribution, we obtain estimates of the parameters of interest, and calculate the level of certainty we ascribe to them. One

advantage of the Bayesian approach is the intuitive interpretation of certainty measures. The "*95% credible interval"* is the range within which we expect, with a probability of 0.95, the true parameter value to be located (Korner-Nievergelt *et al.* 2015). The accuracy of a Bayesian model that uses MCMC improves as the number of MCMC iterations is increased. Early iterations will not be close to the stationary distribution, and a portion of these can be discarded to improve the accuracy of the model, this is the *"burn in"* (Hadfield 2010). Finally, only a portion of the remaining parameter values from each MCMC chain—defined by the *"thinning interval"*—is stored for parameter estimation, to ensure independence of samples from the stationary distribution (Hadfield 2010). Together, the burn in and thinning interval set the total sample size of iterations, that is, the number of MCMC samples that are stored to reconstruct the posterior. In this thesis we use Bayesian modelling via MCMC to implement parentage analyses and GLMMs.

2.5.3 Parentage analysis

Parentage analysis uses genetic data to estimate the relationships between individuals to determine the most likely pedigree (Jones *et al.* 2010). Here we use the R package MASTERBAYES (Hadfield, Richardson & Burke 2006) to conduct a full probability parentage analysis in a Bayesian framework, to reconstruct the pedigree of our *F. ovina* F1 progeny array. Parentage analysis in MASTERBAYES is able to incorporate non-genetic (e.g. phenotypic) information to support the parentage analysis. These parameters are estimated alongside the genetic parameters underpinning the pedigree, allowing more accurate estimates of uncertainty for

both, and reducing bias in estimation (Hadfield, Richardson & Burke 2006). Here we used parentage analysis to determine the paternity of the individuals within the offspring clonal library. Maternal parentage was treated as known, since this was the plant from which the seeds were collected. This information was incorporated within the parentage model to inform the analysis.

Our study population is tetraploid. Since MASTERBAYES does not handle tetraploid genotypes the genetic data were converted to binary data using the *genambig.to.genbinary* function in POLYSAT (Clark & Jasieniuk 2011), and subsequently treated as dominant markers. A final set of error checking was carried out on the genetic dataset to test the accuracy of allele calls. This used a series of random and directed error checks, as well as a preliminary parentage analysis, to identify mis-called alleles (Appendix 2, Section A2.5). The final dataset consisted of genotypes for 553 individuals (457 offspring and 59 parents, including replicate genotypes for 37 of these parents).

2.5.4 MASTERBAYES parentage model

Restrictions were placed on the pedigree via the *Pdataped* argument of *MASTERBAYES*, such that only individuals from the parent offspring library could act as parents, and to specify the maternal parent of each F1 offspring. No other priors were specified and the model was allowed to estimate error rates (E1 = genotyping error, E2 = stochastic error; Hadfield, Richardson & Burke 2006). The model was run for 1,500,000 iterations, with a thinning interval of 700 and a burn in of 800,000, resulting in a sample size of 1,000. We stored the posterior probability distribution

for all parentage inferences with at least 50% confidence level. Autocorrelation of the sample from the Markov chain corresponding to the pedigree was determined using the *autocorrP* function, giving an autocorrelation at lag one of −0.008. Genotyping error rates were estimated as E1 = 0.130 (s.d. = 0.006) and E2 = 3.547×10^{-3} (s.d. = 2.025×10^{-4}). The autocorrelation estimates for E1 and E2 at lag one were 0.323 and 0.090, respectively. The pedigree generated from this model was used for all subsequent analyses. Since samples from the MCMC chain for E1 showed reasonable autocorrelation, we carried out a sensitivity analysis to test the influence of the genotyping error rate estimation on the pedigree (Appendix2, Section A2.6).

2.5.5 GLMM model specifications

GLMMs were fitted in R (R Development Core Team 2008) using the package MCMCGLMM (Hadfield 2010). Unless specified otherwise, all GLMMs were run for 1,300,000 iterations, with a burn in of 300,000 and a thinning interval of 1,000 resulting in a sample size of 1,000. The prior distribution used for variance components in these models was a non-informative uniform improper prior distribution on standard deviation of the random effects (specified as $V = 1.0 \times 10^{-16}$, *nu* = –1 in MCMCGLMM), as recommended by Gelman (2006). Where possible, categorical nuisance parameters (such as experimental blocking factors) were fitted as a set of random effects (when the number of categories was high) or as centred fixed effects (when the number of categories was low). The sensitivity of the model to starting parameters was tested by running each model in triplicate using over-

dispersed starting values. The Gelman-Rubin diagnostic was used to assess convergence (Gelman & Rubin 1992). Autocorrelation was checked within MCMC samples at lag one. A value below 0.1 was required for the level of autocorrelation to be deemed acceptable.

For analyses carried out in MCMCGLMM we report *pMCMC* values, which is a Bayesian equivalent of a p value, and is the probability that the value is significantly different from 0. Model reduction was carried out based on comparison of *pMCMC* values of the parameters of interest and, where appropriate, comparison of the Deviance Information Criterion (DIC). Parameters of interest were extracted as the mean of the posterior distribution for that parameter. *95% credible intervals* were calculated using the *HPDinterval* function in MCMCGLMM. Models were also evaluated by implementing them in in LMER (Bates *et al.* 2015), and then checking for consistency in parameter estimation and precision estimates with the MCMCGLMM version of the model.

2.5.6 Climate-induced differentiation in reproductive success and mating system

We applied GLMM to the number of offspring sired by each paternal parent, and to the number of flowers on each parent to test for phenotypic differentiation between climate treatments at BCCIL. All 59 parent plants were included in the analysis, even those that did not sire any offspring. The number of offspring sired were fitted with a Poisson family. The number of flowers were log transformed, and fitted with a Gaussian family. To test whether the number of offspring sired was related to the number of flowers on that plant, we analysed the data with a GLMM,

fitted with a Poisson family. In each of these analyses, the plot of the paternal parent at BCCIL was fitted as a centred fixed effect.

We chose to assess whether patterns of mating were assortative, that is mating in which mating individuals have phenotypes that are more similar than would be expected under random mating (Fox 2003; Bos & Caligari 2008). Such non-random mating may contribute to reproductive isolation among *F. ovina* sub-populations from different climate treatments at BCCIL, potentially increasing the rate of evolutionary responses to the climate. We used the pedigree to test for assortative mating among parent individuals originally collected from BCCIL.

We used a Pearson's chi-squared test to determine whether assortative mating favoured mating between pairs of plants that originated in the same climate treatments at BCCIL. We also used a permutation test to determine whether there was assortative mating with respect to flowering time (i.e., whether pairs of plants with similar flowering times were more likely to mate than other pairs of plants). Flowering time data were taken from a separate experiment in which replicates of each of the plants from the parent clonal library had been grown in a common garden environment under standardised conditions, at Ness Botanic Gardens. Flowering time data were collected during the summer of 2013 and flowering time was measured as the date of first anthesis (at which the first anther is visible in any spikelet; further details of this method presented in Appendix 2, Section A2.7). The maximum difference in first flowering date between any pair of plants was 17 days, but most of the flowering times (56 out of 58) fell within a ten-day interval. To carry out the permutations test we first constructed a matrix of the dissimilarity in flowering time between all possible parent pairs (excluding selfing; for n = 58

parent plants). A single parent plant (and a mating event associated with its single progeny) was excluded from the analysis because it had no flowering time information. We observed 434 remaining mating events among parent individuals, thus we drew 99,999 random samples of this size from the matrix of flowering time dissimilarity. For each draw from the matrix, we calculated the mean flowering time dissimilarity, summarising the tendency for plants to mate according to flowering time. The observed value for the mean flowering time dissimilarity was also added to this set, to give 100,000 values in total. Together, these draws represent a null distribution for flowering time dissimilarity, defining the expectation if mating were at random. The observed mean value of the flowering time differences was compared to the null distribution to test whether mating was random with respect to flowering time.

2.5.7 Germination of offspring

The probability of whether or not a seed germinated was analysed with a GLMM on the germination data of 914 seeds. The date of germination and final observation were standardised to take into account slight differences in preparation dates. The data were fit with a binary family. The residual variance was fixed at 1. Seed mass and maternal ancestral climate at BCCIL were fitted as fixed effects. Maternal clone was fitted as a random effect. No paternal information could be fitted for this model.

Paternity assignments supported with a probability of at least 0.5 were made for 435 offspring. Of those seeds that germinated and were planted, 431 had

their parentage assigned with at least 0.5 probability. The *ancestral climatic environment* at BCCIL for both parents of an individual seed could be determined using these most probable paternity assignments and resulted in the progeny array described in Table 2.2.

Table 2.2 The paternity assignments of the F1 progeny array

Mother		**Father**	**Offspring**	**N**
Control	×	Control	Pure control ancestry	135
Control	×	Drought	Hybrid ancestry	208
Drought	×	Control		
Drought	×	Drought	Pure drought ancestry	88

We used GLMM to test for evolutionary change in seed mass driven by drought treatment at BCCIL. Parental climate ancestry at BCCIL was fitted as a fixed effect with three levels (Table 2.2; *pure control*, *hybrid* and *pure drought ancestry*). Maternal and paternal clone were fitted as random effects.

We used survival analysis to examine the timing of germination to climatic selection using the R package SURVIVAL (Therneau & Grambsch 2000). Survival analysis is used to analyse the time to an event, typically death; however, here, the response is time from seed imbibition until germination. The date of germination and final observation were standardised to take into account slight differences in preparation dates. We fitted a parametric survival regression model with parental ancestry and seed mass as explanatory variables. We tested an exponential error distribution for this model (with a constant hazard of germination) and a Weibull error distribution (with a non-constant hazard of germination). The Weibull error distribution gave a significantly better-fitting model, so this parameter was used in

the model testing. The best fitting model was assessed by comparison of the Akaike Information Criterion (AIC) values.

2.6 Results

2.6.1 Microsatellite genotyping and paternity analysis

A total of 553 *F. ovina* individuals were genotyped successfully at one or more microsatellite loci. 518 individuals (93.7% of the total) were represented by genotypes at 8 or more of the 9 loci (comprising 55 of 59 parents, 93.2%; 36 of the 37 replicated samples, 97.3%; and 427 of the 457 offspring, 93.4%). The number of alleles per locus ranged from 6 to 20 (Table 2.3).

Table 2.3. *Festuca ovina* microsatellite marker and population genetics summary statistics.

Marker	Total No. alleles	No. of individuals scored
T_02	11	546
T_06	19	527
T_19	20	505
T_23	16	530
T_25	10	547
T_26	6	504
T_28	16	550
T_35	9	462
T_42	17	474

The total multi-locus $P_{(ID)}$ was calculated as 2.861×10^{-5}, which means that there is a very small probability that two individuals selected at random from the population will have the same genotype. Each parental clone was separated from all other parent clones by at least 0.2 *meandistance.matrix2* distance, and hence can be distinguished as genetically unique.

Paternity assignments with a probability of 1 were made for 249 out of 457 of the *F. ovina* F1 offspring (54.5 % of the total). 397 (86.9 %) individuals had paternity assignments with a probability of greater than 0.8, and 435 (95.2 %) of individuals had paternity assigned with a probability of at least 0.5.

2.6.2 Climate-associated differentiation in mating system

For the analyses of reproductive success we used 435 offspring that were assigned parentage with a probability of at least 0.5. Of the 59 parent plants, 54 successfully sired offspring. The number of offspring sired by a single father ranged from 0 to 26.

There was a significant difference in the number of offspring sired based on the ancestral climate treatment of the father (*pMCMC* < 0.048). Fathers from drought plots sired significantly fewer offspring than fathers from control plots, with plants siring an average of 7.0 compared to 4.4 offspring from control and drought plots respectively (Figure 2.2 **A**). There was no significant difference between the number of flowering tillers on the plants in 2012 dependent on the treatment the plant was under at BCCIL (Figure 2.2 **B**; *pMCMC* = 0.188). The number of offspring sired was significantly positively correlated with the number of flowering tillers on the plant (Figure 2.2 **C**; *pMCMC* < 0.001).

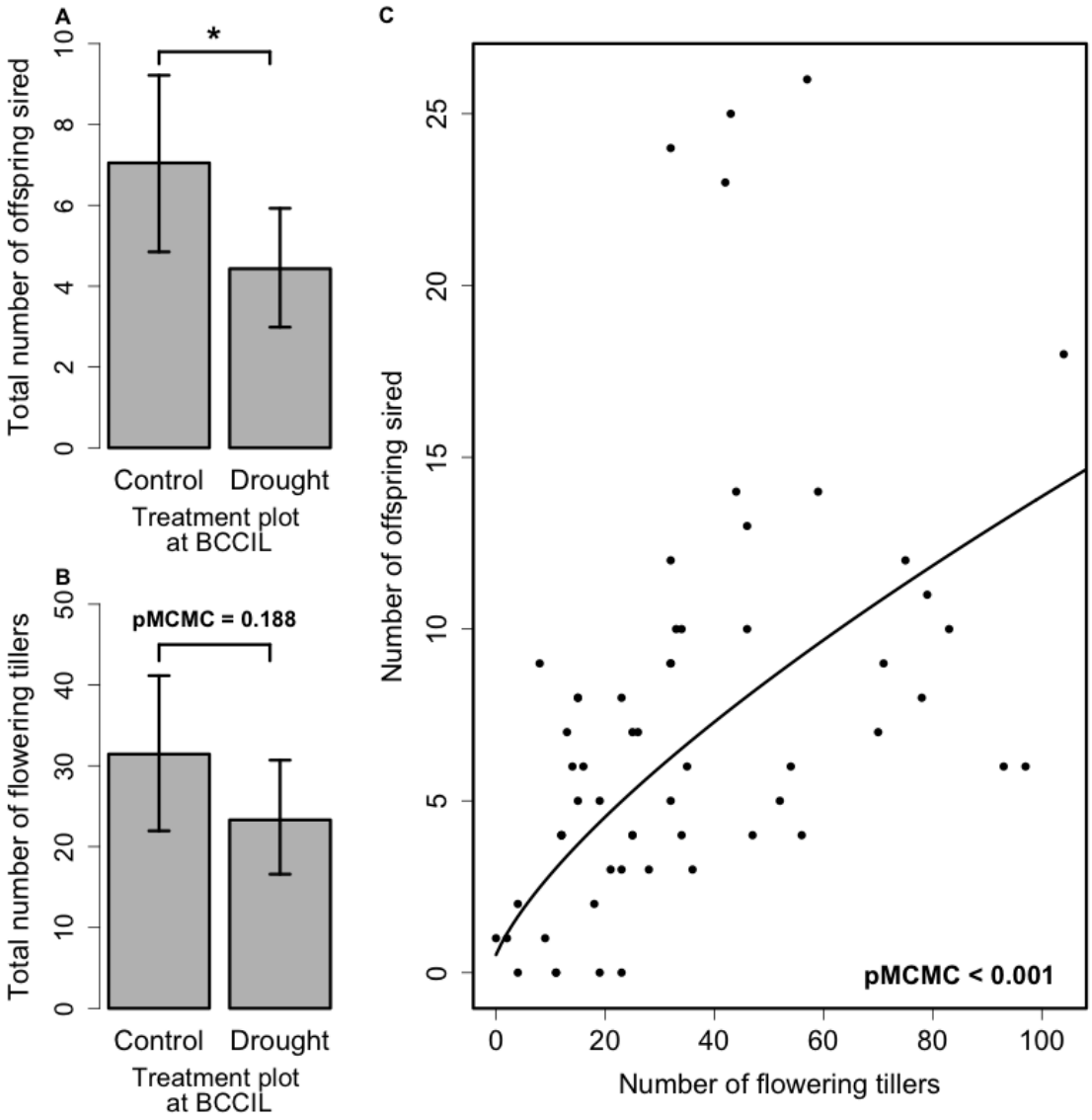

Figure 2.2 Evolutionary differentiation in reproductive success between *F. ovina* populations from different climate treatments at BCCIL. **A)** Male reproductive success, measured as the number of offspring sired per parent plant. **B)** Female reproductive success, measured as the number of flowering tillers per parent plant. **C)** Relationship between male and female reproductive success, measured as above. The curve represents the predicted relationship between male and female reproductive success, estimated from the MCMCGLMM model output. Error bars represent 95% credible intervals. Significance levels: * *pMCMC* < 0.05; ** *pMCMC* < 0.01; *** *pMCMC* < 0.001.

There was no evidence of assortative (non-random) mating with respect to climate treatment of origin at BCCIL (χ^2 = 0.642; d.f. = 1; p = 0.422). Plants from the same treatment were not more likely to mate with each other (Figure 2.3 **A**). Mating was assortative with respect to flowering time (Figure. 2.3 **B**). Under random mating we would expect to see a mean difference in flowering time of 2.77 days, we observe a mean difference in flowering time of 2.46 days.

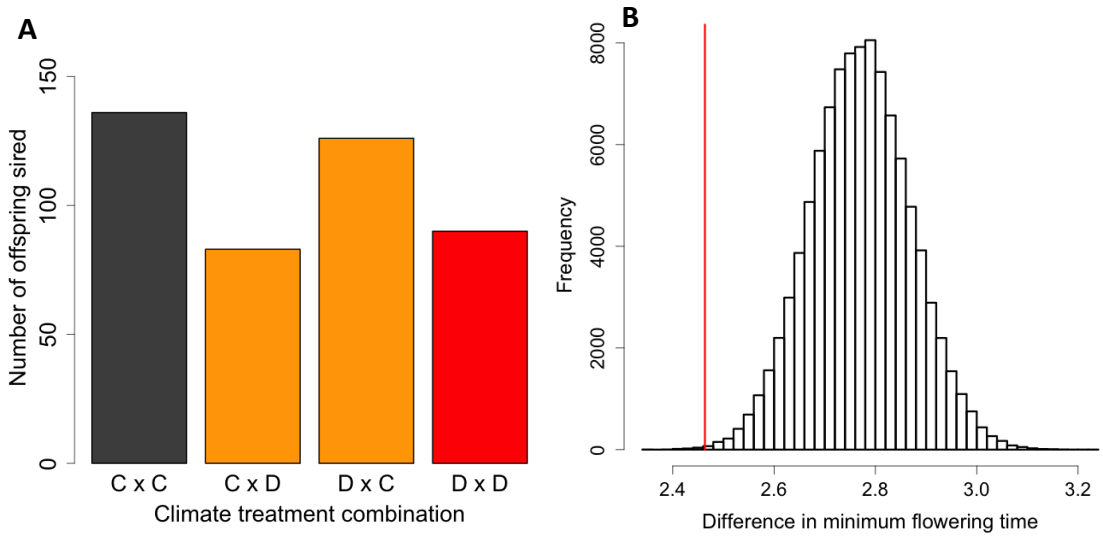

Figure 2.3 Patterns of assortative mating **A)** The number of offspring sired with respect to climate treatment of origin at BCCIL. C = Control, D = Drought **B)** A histogram of the null distribution of mean differences in flowering time following 99,999 samples of 434 from a 1,653 matrix containing all of the possible differences in flowering time. The red line shows the location of the mean difference in flowering time that we observe in the parent clonal library.

2.6.3 Probability of germination

In total, 92.5% of all seeds germinated. The average seed mass was 0.745 mg. Germination probability was significantly predicted by seed mass (*pMCMC* < 0.001), with heavier seeds more likely to germinate (Figure. 2.4). The average mass

of germinating seeds was 0.770 mg (n = 846) while the average mass of seeds failing to germinate was 0.424 mg (n = 68). There was no significant difference in seed mass between F1 seeds with pure control ancestry (both parents originating from control plots) and those of pure drought ancestry (*pMCMC* = 0.652).

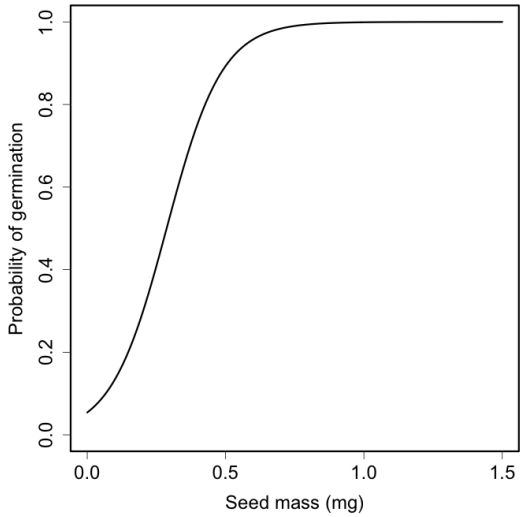

Figure 2.4 The probability of germination dependent on seed mass calculated from the Bayesian model re-run with seed mass as single fixed effect.

Seed mass and ancestral climate both had significant effects on lag to germination (survival analysis; seed mass effect, $p < 0.001$; climate effect, $p < 0.001$; Figure 2.5). Mean lag to germination in seeds with pure drought ancestry was 2.8 days greater than for those seeds with pure control ancestry (Figure 2.5 **A** & **B**). Seeds with hybrid climate ancestry showed intermediate germination latency (mean germination latency for seeds with control ancestry = 10.2 days, with hybrid ancestry = 11.4 days, with drought ancestry = 13.0 days). Seed mass was negatively associated with the number of days to germination (Figure 2.6 **D**), and this effect was driven in large part by the late germination of seeds with the smallest masses (Figure 2.5 **C**). The best fitting survival model for germination latency incorporated a non-constant hazard of germination, via a Weibull error distribution, implying that the hazard of germination decreased with time in our study population.

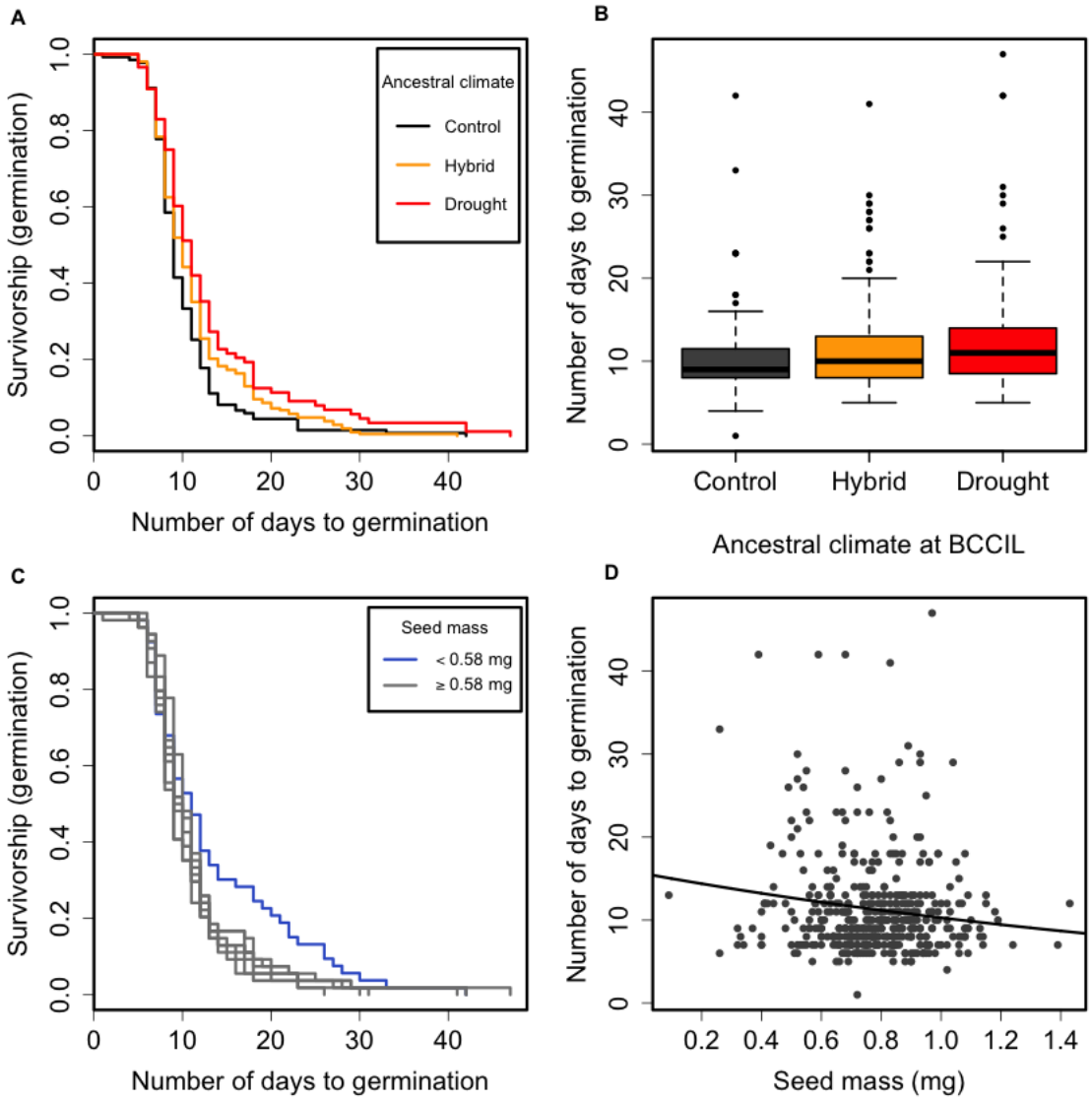

Figure 2.5 Responses of germination latency to climate ancestry and seed mass **A)** Kaplan–Meier plot showing predicted germination schedules for seeds with ancestry in different climate treatments at BCCIL. **B)** Boxplot summarising germination latency data by parental ancestral climate. The box represents the first and third quartile, the whiskers extend to ± 1.5 × interquartile range, and points lying outside the range of the whiskers represent outliers. **C)** Kaplan–Meier plot showing predicted germination schedules for seeds in different mass categories. The seed masses were grouped into 8 categories with approximately equal sample sizes: 0.09–0.57 mg, n = 53; 0.58–0.67 mg, n = 54; 0.67 mg – 0.72 mg, n = 54; 0.72 mg – 0.78 mg, n = 54; 0.78 mg – 0.84 mg, n = 54; 0.84 mg – 0.89 mg, n = 54; 0.90 mg – 0.97 mg, n = 54; 0.97 mg – 1.43 mg, n = 54. **D)** The lag to germination by seed mass. The trend line is the relationship between seed mass and germination latency estimated from the SURVIVAL model. Each data point represents the germination time and seed mass for a single seed.

2.7 Discussion

In this chapter we developed and used microsatellite markers to establish an F1 progeny array from clonal lines of *F. ovina* collected from BCCIL, via pedigree analysis. We then used this progeny array to determine whether simulated climate change treatments at BCCIL have driven evolutionary changes in mating system characteristics and seed germination traits. We have found that long-term drought treatment has driven correlated decreases in male reproductive success (measured as progeny sired) and potential female reproductive success (measured as number of flowering tillers). We find evidence for evolutionary change in germination time, with greater delays to germination occurring in plants whose parents both originated from the drought treatment at BCCIL. Furthermore, we have shown that mating is assortative by flowering time, which could alter the speed of evolutionary responses and provide a route to partial reproductive isolation of populations occupying different climate treatments at BCCIL. Taken together, these results suggest climate-driven evolutionary changes to critical life-history traits within *F. ovina*.

2.7.1 Reproductive success

Our results demonstrate that male reproductive success has decreased in response to long-term experimental drought manipulations at BCCIL by 37.1% relative to control plants. Plants from the drought treatment also produced fewer flowers than those from the control treatment, and the number of offspring sired was correlated with the number of flowering tillers the plant produced in that year.

Thus, a likely explanation for the reduction in male fitness in plants from the drought treatment is that plants with fewer flowers have a lower total quantity of pollen, reducing the chance that pollen is transferred to a receptive stigma of another plant. However, we cannot rule out the possibility that other aspects of male function, such as pollen tube growth rate and pollen germination, have also responded to drought, contributing to the observed reduction in male reproductive success (Schaeffer, Manson & Irwin 2013). If other fitness components, such as female reproductive output and survival, do not compensate for this reduction in male fitness, then plant total fitness will be reduced, leading to an increased risk of population extinction under climate change.

Flowering tiller production and male reproductive success showed a positive relationship, but there was considerable residual variation in male fitness about this trend (Figure 2.2 **C**). In particular, four plants showed reproductive success far greater than expected based on the number of flowers they produced. This finding emphasises the need for direct measurements of male reproductive success, and suggests that the often-used assumption that flower number is a reasonable proxy for this trait may be invalid at the level of plant individuals (Snow & Lewis 1993). There was a trend of diminishing gain in offspring sired with increasing number of flowers. This pattern has been predicted in theory (Willson & Rathcke 1974; Burd & Callahan 2000), and observed in some experimental studies (Schoen & Stewart 1986; Broyles & Wyatt 1990; Devlin, Clegg & Ellstrand 1992; Karron & Mitchell 2012). However, most of these studies are on insect pollinated species where the mechanisms controlling the relationship between flower number and seeds sired will be very different from those found in wind-pollinated species such as *F. ovina*.

A more relevant comparison can be made with the study by Schoen & Stewart (1986) on wind pollinated *Picea glauca* (White Spruce), who found that the number of cones a plant produced was correlated with the proportion of seeds that the plant sired, and that the gain in offspring sired diminished at a very high number of cones.

The phenotypic differentiation in male reproductive success and number of flowers that we have observed here were in plants taken directly from the field, grown in a common environment. These responses may be carry-over from the field, as opposed to genetic change necessary to demonstrate evolution. However, in Chapter 3, we demonstrate that inflorescence number has significant, though low levels of heritable genetic variation, which strengthens the case for these responses being evolutionary. These results provide a novel demonstration of the potential negative impact of climate change on male reproductive success.

2.7.2 Assortative mating

Assortative mating can increase the speed of evolutionary responses and provide a mechanism for partial reproductive isolation within populations (Fox 2003; Weis *et al.* 2005). We found no evidence that plants that originated from the same climate treatment are more likely to mate with each other. However, we did find evidence for assortative mating that favoured plants with coincident flowering times. This is important, because in a separate common garden experiment, the *parent microcosm experiment* (on the parent clonal library), it has been shown that plants from the drought treatment flower significantly earlier than those from the

control treatment. Together, these results suggest that over time we might expect to see the evolution of partial reproductive isolation between plants from drought and control treatments at BCCIL. A similar situation has occurred in the long-term experimental treatment plots at the Park Grass Experiment, in Rothamsted. Here, Snaydon and Davies (1976) found that different nutrient treatment regimes have driven evolutionary differentiation in the flowering time of the short-lived grass *Anthoxanthum odoratum*. Silvertown *et al.* (2005) later demonstrated that selection on flowering time had reinforced reproductive isolation between the plots. Another well-studied example of assortative mating was discovered in the annual plant *Brassica rapa*, where it has been shown that genetic variation in flowering time has led to assortative mating (Weis & Kossler 2004). Franks & Weis (2009) found that the evolution of flowering time in *B. rapa* in response to a 5-year natural drought altered reproductive isolation between populations through phenological assortative mating. Our results provide very strong evidence for assortative mating by flowering time in a long-lived perennial plant, and suggest the potential for reproductive isolation under experimentally imposed chronic drought treatment.

Our experiment was intended to generate a set of sexual F1 offspring with a known pedigree, to support the remaining work in this thesis. Thus, our design was balanced with respect to the number of seeds from each maternal plant that were planted and used. Unfortunately the total quantity of seed produced by each parental plant was not measured. Hence, we cannot compare the relative contribution of male and female reproductive success to total fitness. Nonetheless, our results provide strong support to the hypothesis that plant mating systems

evolve under climate change, and that such changes may act to reinforce evolutionary changes occurring in other traits.

2.7.3 Germination of offspring

Our results suggest that differences in germination timing have evolved in *F. ovina* in response to long-term drought manipulation at BCCIL. Seeds with pure control ancestry were on average quicker to germinate than those with hybrid ancestry or pure drought ancestry. The ancestral climate of F1 seeds also affected the shape of the germination latency curve, with the offspring of pure control ancestry having a smaller range in lag to germination than offspring descended from drought-treated plots. Parental ancestral climate had no effect on the seed mass, but seed mass was associated with germination latency; lighter seeds had on average a longer time to germination. The differences in germination timing that we have observed here were measured on offspring traits under common environmental conditions, following the growth of parent plants in a common environment for 3 years prior to the collection of seed. This provides a strong case for the differences we observe being the result of genetic change and thus evolutionary responses (as opposed to carry over effects from the field). However, some studies have suggested that seed traits, particularly seed mass, are so strongly influenced by *maternal effects* that they can be viewed as a phenotype of the mother (Thiede 1998; Galloway, Etterson & McGlothlin 2009). Therefore, there may still be a component of carry-over effect from the field, despite our best efforts to reduce its effect.

To understand climatic selection on lag to germination, it is helpful to understand the context of germination in the field. An extensive study of the seasonal timing of seed germination in natural populations of *F. ovina* in Northern England was carried out by Thompson & Grime (1979). They found a well-defined seasonal pattern of seed germination in *F. ovina*, with seeds falling from the plant during June through to September. Seeds were observed to germinate soon after, from August to December (Figure. 2.6). Thus, Thompson and Grime concluded that *F. ovina* seeds take advantage of bare ground left by disturbance in the grassland following summer droughts and animal grazing to initiate germination (Thompson & Grime 1979; Grime *et al.* 1981).

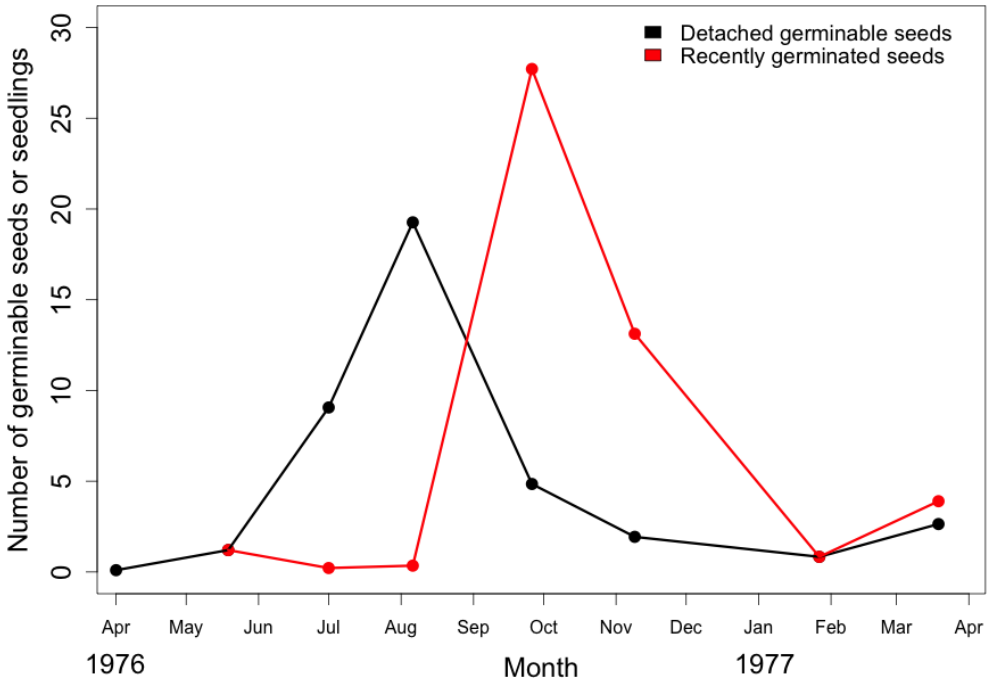

Figure 2.6 Variation in numbers of germinable seeds or seedlings in *F. ovina* during the period from April 1976 to March 1977, reproduced from Figure 8; Thompson and Grime (1979) using WEBPLOTDIGITIZER version 3.1 (Rohatgi 2016).

The ability of *F. ovina* to germinate under a wide range of temperatures facilitates germination during the end of summer and autumn when temperatures may be variable. These natural seasonal dynamics set a context in which a longer delay to seed germination under drought conditions could be advantageous. First, the drought treatment at BCCIL runs through July and August; the first precipitation following the drought is likely to initiate seed germination. Soil moisture at this time could still be very low, although it usually recovers quickly (Fridley *et al.* 2011), and established plants that have survived the drought may quickly take up much of the water from the first rain. Therefore, a longer lag to germination may be adaptive to lengthen the time available for further precipitation to replenish soil moisture, enabling optimum conditions for germination. Secondly, we know that plants from the drought plots flower significantly earlier than plants from the control plots. Our own observations suggest that seeds from these plants mature earlier and fall from the plant slightly sooner. The lengthened lag to germination may simply bring the timing of seedling emergence back in line with that found under non-drought conditions. Alternatively, a lag to germination, may be selecting against germination during the drought itself, as it may be that during the drought moisture is available in the form of condensing water from fog or cloud. We do not know which of these options is more likely, or know whether the lag in germination that we observe in the lab is representative of those expressed under field conditions. Monitoring seed maturation, germination, seedling establishment and survival in the field at BCCIL would establish whether and how evolutionary changes in germination timing are adaptive under field conditions.

An alternative explanation for the evolutionary response in germination timing is that this change was the result of correlated evolution with another trait. For example, artificial selection for earlier flowering time in the plant *Campanulastrum americanum* resulted in the correlated evolution of delayed germination time (Burgess, Etterson & Galloway 2007), implying that these traits may share part of their genetic basis. Studies in *Arabidopsis thaliana* have also revealed pleiotropy between flowering time and germination. Specifically, the FLOWERING LOCUS C (FLC) gene is strongly associated with both traits (Chiang *et al.* 2009; Debieu *et al.* 2013). Association between flowering time and seed dormancy has also been demonstrated in *Capsella bursa-pastoris* (Toorop *et al.* 2012). In our study, we do not know whether flowering time, or germination lag, is the direct subject of selection, and which may be the result of pleiotrophic effects, but in either case our results support climate-driven evolutionary change. Correlated evolution between traits under climatic selection may produce unexpected responses in the other traits, which may reinforce or disrupt adaptive evolution under an altered climate.

2.7.4 Conclusions

Together, our results suggest that the plant mating system and early-acting life-history traits in *F. ovina* are evolving in response to long-term climate change treatments at BCCIL. Male reproductive success has been reduced in plants from the drought treatments, reflecting changes in flower number. However, we do not know whether other components of fitness have evolved to compensate, or

whether this response is maladaptive. Our finding of assortative mating by flowering time indicates a mechanism by which partial reproductive isolation could develop between plants from the drought-treated and control treatments at BCCIL, potentially reinforcing the development of climate-driven adaptive evolution. We also found that a greater lag to germination has evolved in the *F.ovina* population from drought-treated plots at BCCIL, although we do not yet know whether this response is adaptive or the result of correlated evolution. These results suggest that plants are capable of rapid evolutionary change, which may provide a mechanism by which they can persist through climate change.

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3 Chapter 3. Heritability, genetic architecture and constraints on evolutionary responses to climate change in *Festuca ovina*

3.1 Abstract

The evolutionary potential for adaptation to climate change depends on the genetic architecture underpinning climatically adaptive traits: in particular, the presence of heritable genetic variation, and the extent to which different traits share a genetic basis. Genetic trade-offs between traits that are selected under climate change and other ecologically important traits may limit adaptive evolutionary responses. Thus, the assessment of genetic architecture is critical for understanding the potential for evolution to buffer populations from the effects of climate change. Here, we describe the genetic architecture of a population of *Festuca ovina* collected from grassland plots exposed to chronic drought treatment and from corresponding control plots, at the Buxton Climate Change Impacts Laboratory (BCCIL). We measured the phenotypes of 449 F1 progeny derived from field-collected *F. ovina* with known pedigree, in a common garden environment. We report significant heritable genetic variation in morphological and reproductive plant traits (h^2 = 0.014–0.300). We also show that there is a strong maternal component to phenotypic variation within this population. Quantitative genetic analysis revealed positive genetic correlations between plant biomass production and leaf size and mass, reflecting genetic differences in plant size. We also document a negative genetic correlation between asexual reproduction (tiller

number) and reproductive traits (number of seeds). This genetic architecture suggests an evolutionary trade-off between, on the one hand, asexual propagation that may underpin survival and, on the other hand, reproductive performance. These results imply that evolutionary responses to climate change will not be able to increase simultaneously sexual, asexual and competitive components of fitness. Finally, growth patterns in the common garden environment did not support evolutionary differentiation between plants with bi-parental ancestry in the drought or control treatments at BCCIL.

3.2 Introduction

Climate change is rapidly altering the environmental conditions experienced by plants across the globe, altering selection pressures on populations (Shaw & Etterson 2012). Evolutionary responses may provide a way for plants to adapt to changing conditions and persist (Hoffmann & Sgro 2011; Franks, Weber & Aitken 2014); such rapid evolution in response to climate change has now been documented in several plant species (Franks, Sim & Weis 2007; Nevo *et al.* 2012; Thompson *et al.* 2013; Ravenscroft, Whitlock & Fridley 2015). The potential for adaptive evolutionary responses depends, in part, on the genetic architecture underlying traits, defined as the structure of genetic variance and covariance within and among traits (Hoffmann & Sgro 2011; Shaw & Etterson 2012). Quantitative genetic studies can help us to understand the capacity for populations to evolve in response to climate change, through quantification of the genetic variance and

heritability, and assessment of evolutionary constraints arising from antagonistic genetic correlations between advantageous traits (Anderson *et al.* 2014; Gienapp & Brommer 2014; Kremer, Potts & Delzon 2014). Thus, these approaches can make a vital contribution to our understanding of the role of evolution in underpinning plant population persistence during climate change.

The presence of heritable standing genetic variation within a population is a prerequisite of evolution by natural selection (Hoffmann & Merilä 1999). Measures of narrow sense heritability (the proportion of phenotypic variance explained by additive gene effects) provide an indication of the degree to which a trait can evolve under selection (Lynch & Walsh 1998). However, estimates of heritability can vary both among environments and among populations (Mazer & Schick 1991; Donohue *et al.* 2000; Conner, Franks & Stewart 2003; Etterson 2004). This means that the evolutionary potential of a population will vary depending on the environmental context and the particular population under study.

The presence of heritable, quantitative genetic variation does not necessarily mean that evolutionary responses to climatic selection are a foregone conclusion. Central to life-history theory is the understanding that trade-offs between traits can impede evolution; multiple traits cannot all evolve to optimise fitness (Stearns 1992; Sgrò & Hoffmann 2004; Roff & Fairbairn 2007). In other words, selection does not act on traits in isolation from each other, because different traits often share a proportion of their genetic basis. Such genetically correlated traits lead to correlated responses to selection (Lynch & Walsh 1998; Etterson & Shaw 2001). A genetic correlation is the proportion of variance shared by two traits due to additive genetic causes (Lynch & Walsh 1998). Genetic

correlations are the result of either pleiotropy, a single gene affecting the phenotype of more than one trait, or linkage disequilibrium, the non-random association of alleles at different gene loci (Lande 1980; Lande 1984; Lynch & Walsh 1998). The direction and strength of a genetic correlation between two traits can constrain or expedite evolutionary responses (Lynch & Walsh 1998). Evolutionary responses will be facilitated where selection pressure is in a direction parallel to the correlation between traits (Figure 3.1 **A**). In contrast, evolutionary responses will be constrained when selection pressure is perpendicular to the genetic correlation (Etterson & Shaw 2001). For example, where two traits are strongly positively correlated, but selection favours an increase in one trait value and a decrease in the other trait value (Figure 3.1 **A**), or where two traits are strongly negatively correlated, but selection favours an increase in the values of both (Figure 3.1 **B**), evolution will be impeded (Etterson & Shaw 2001). In a study of *Lobelia,* Caruso *et al.* (2005) found that the constraints to evolutionary responses imposed by underlying genetic architecture differed between *Lobelia* species studied. In *L. cardinalis* they found that traits related to water use had little genetic variation, which suggested potential limits to adaptation to drier conditions. In contrast they found that *L. siphilitica* had significant genetic variation in all of the traits studied, but there was a significant negative genetic correlation between plant size and water use efficiency. The authors argued that such genetic architecture would be likely to constrain the evolution of both stress tolerance and increased sexual fitness under drought conditions. Etterson & Shaw (2001) and Etterson (2004) found that adverse genetic correlations among traits and across environments were likely to limit climate-driven adaptive evolutionary responses in *Chamaecrista*

fasiculata, despite the presence of sufficient genetic variation. Temporal climatic variation among plant generations can further complicate evolutionary responses to underlying directional climatic selection. In this case, a different subset of individuals would be selected in different years, slowing the evolutionary response to long-term directional climate change (see Figure 1 in Etterson 2004). A sound understanding of the genetic architecture underpinning plant phenotypes can help us to understand the potential for, and limitations of, adaptive evolutionary responses to climate change.

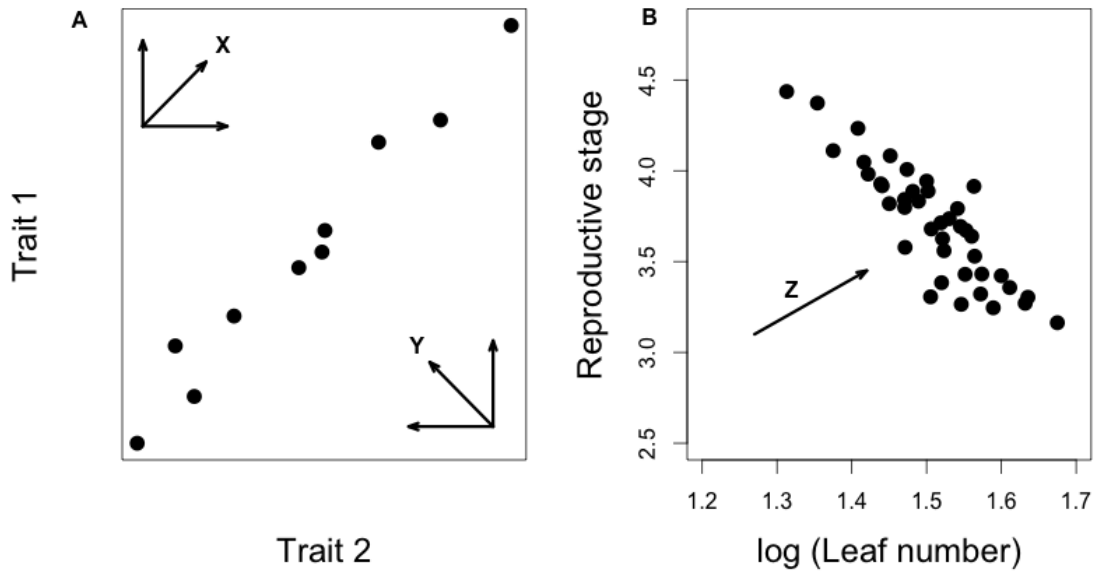

Figure 3.1 Examples of the way the genetic correlations can constrain evolutionary responses, dependent on the direction of selection. **A)** An example of a theoretical positive genetic correlation between two traits. In scenario **X**, the direction of selection matches the direction of the genetic correlation, facilitating an evolutionary response. In scenario **Y**, the direction of selection is perpendicular to the direction of the genetic correlation, and evolutionary responses may be constrained. **B)** An example of a negative genetic correlation between two traits. In scenario **Z**, the direction of selection is perpendicular to the genetic correlation, and evolutionary responses may be constrained. Reproduced from Figure 1; Etterson & Shaw (2001) using WEBPLOTDIGITIZER version 3.1 (Rohatgi 2016).

In this chapter, we examine how the genetic architecture underlying plant phenotypes may influence evolutionary responses to climate change in the perennial grass *Festuca ovina*. We use individuals of *F. ovina* collected from the Buxton Climate Change Impacts Laboratory (BCCIL) study system. At BCCIL natural calcareous species-rich grassland has been subjected to long-term experimental climate change treatments for 17 years. Natural populations of *F. ovina* typically show high levels of genetic variation in quantitative traits, and work specifically on the BCCIL population has shown that these traits have broad-sense heritability (Bilton *et al.* 2010, R. Whitlock, personal communication). In *the parent microcosm experiment*[3], in which clonal replicates of *F. ovina*, collected from BCCIL, were grown in a common environment, it was found that the populations of *F. ovina* from different climate treatments have diverged phenotypically. Furthermore, AFLP genotyping applied to *F. ovina* populations from different climate treatments at BCCIL also revealed significant genetic differentiation between climate treatments (Ravenscroft, Whitlock & Fridley 2015). Together, these findings strongly suggest that evolutionary responses are occurring in response to climate change at BCCIL. However, in order to establish whether such phenotypic and genetic responses are consistent with evolutionary change, it is necessary to determine whether climate-responsive phenotypes are heritable in the narrow sense, i.e. whether selected phenotypes can be passed on to future plant generations. In addition, we know nothing regarding the extent to which genetic architecture within the BCCIL population of *F. ovina* may constrain or facilitate evolutionary responses to climatic

[3] Words in italics are defined in the glossary

selection. In this chapter we estimate the heritability and genetic architecture in a population of *F. ovina* from BCCIL, to identify the potential for, and possible constraints on, evolutionary responses to climate change. We also examine whether there is phenotypic differentiation between plants from different climate treatments at BCCIL, which would indicate an evolutionary response to climatic selection.

3.3 Methods

3.3.1 Study system

This work uses *F. ovina* collected from the BCCIL study system (see Chapter 1, Section 1.4 for further details on BCCIL). In brief, at BCCIL plots of calcareous, species-rich grassland have been subjected to experimental climate change since 1994 (Grime *et al.* 2008). Climate treatments comprise summer drought, winter warming, supplementary summer rainfall (via watering), and factorial combinations of these (drought + warming, warming + watering). Plots are replicated five times in a randomised block design including control plots. Here, we focus on the two-month summer drought treatment—imposed using rain shelters during July and August each year—and control plots. The drought treatment at BCCIL leads to significant reduction in soil-surface water potential: -1100 kPa in drought plots, compared to -20 kPa in control plots by the end of the treatment period (Fridley *et al.* 2011). The greatest changes in species composition at BCCIL have occurred under experimental drought, which has driven reorganisation of the community

structure along a fine-scale soil-depth gradient present within each plot. The drought treatment has also reduced biomass productivity of the sward (Grime *et al.* 2008; Fridley *et al.* 2011). *Festuca ovina,* the focal study species of this thesis, has increased in abundance in the drought plots. In the *parent microcosm experiment*, in which clonal replicates of *F. ovina*, collected from BCCIL, were grown in a common environment, it was found that the populations of *F. ovina* from the drought plots at BCCIL have an earlier flowering time, less reproductive effort and a smaller specific leaf area, relative to individuals from control plots.

3.3.2 Festuca ovina plant material

In July 2010 30 individuals of *F. ovina* were collected from both drought and control plots at BCCIL, by R. Whitlock, using a random stratified sampling design (six individuals per plot, per treatment, 60 individuals in total; see Chapter 2, Section 2.3.2 for a detailed description). One individual subsequently died leaving 59 plants. These individuals are maintained as clonal lines at Ness Botanic Gardens, hereafter the *parent clonal library*. Maintenance of these clonal lines involves inflorescence removal during the summer, biomass clipping in September, weeding, and supplementary watering as required.

During the flowering period of 2012 (May-June) the individuals in the parent clonal library were allowed to mate through natural wind pollination. Plants were systematically moved during the flowering period to minimise spatial effects on patterns of mating. 58 of the parent plants produced seed and 16 seeds from each maternal parent were selected at random and germinated on filter paper in petri

dishes (full details provided in Chapter 2, Section 2.3.3). Eight seedlings were selected at random from those seeds that germinated, and were planted on into seed trays (24 cell trays, each pot 5 cm × 5 cm × 5 cm). These individuals are maintained as clonal lines at Ness Botanic Gardens, hereafter the *offspring clonal library*. Maintenance of clonal lines within the offspring clonal library was the same as for the parent clonal library. Thus, clonal tiller production was the only mode of plant growth in both the parent and offspring clonal libraries.

3.3.3 Common environment experiment

In January 2013 four tillers of comparable size were removed from each individual in the parent and offspring clonal libraries. Each group of tillers was transferred to an individual cell within a 6 × 4 cell tray, each cell 5 cm × 5 cm × 5 cm (LBS Horticulture), filled with a 1:1 mixture of natural rendzina soil (collected adjacent to the BCCIL site) and Perlite (LBS Horticulture). Clones of the 59 parent plants and 464 offspring plants were planted in a randomised blocked design comprising 8 blocks. Each block contained one randomly selected offspring from each of the 58 maternal parents that produced seed (i.e. 8 offspring per maternal parent plant, with the exception of one maternal family which was represented by 7 offspring, and another which comprised 9 offspring). Planting locations for the 59 maternal parent plants were selected at random across blocks. Tillers were "standardised" by cutting and removing leaf tissue above 25 mm; root tissue was not standardised. The cell trays were located outside in a raised experimental bay at Ness Botanic Gardens (Figure 3.2); thus plants were exposed to ambient weather

conditions. Watering was used to supplement rainfall only if there was no precipitation for approximately three days (June-August), or four days (March-May & September), in which case each pot was watered with 25ml of deionised water. During the months of October to February low temperatures meant additional watering was not required, even during periods with little rain. The experiment was maintained from January 2013 until August 2015.

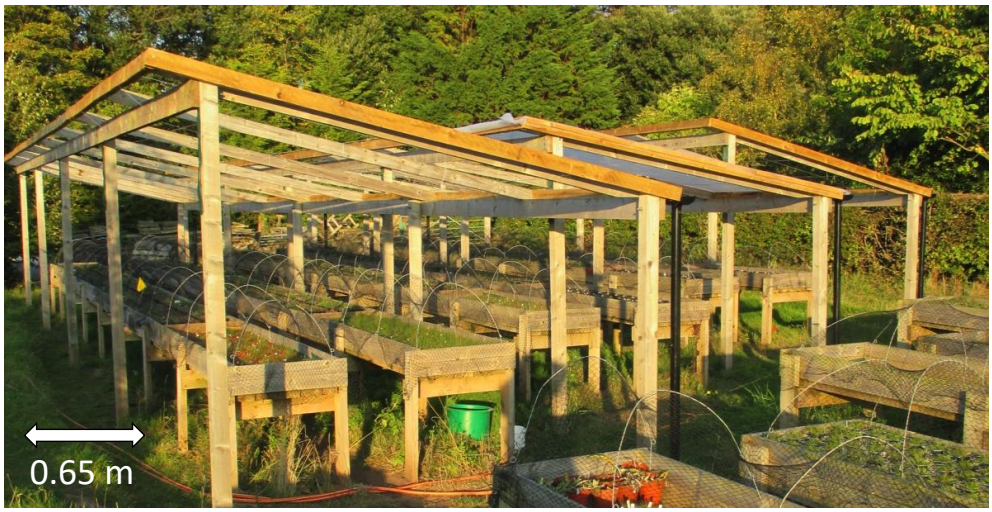

Figure 3.2 The purpose-built experimental bays at Ness Botanic Garden, University of Liverpool, UK where the parent clonal library and offspring clonal library were housed and maintained. The bays are raised above the ground to prevent rabbit damage and are covered by netting to prevent damage by birds.

3.3.4 Trait measurements

We chose to study traits that are important components of plant fitness, or that are useful for assessing plant life-history strategy at the species level (Wilson, Thompson & Hodgson 1999). The following traits were measured: leaf length, leaf width, leaf surface area, leaf wet mass, leaf dry mass, tissue density, specific leaf

area (SLA), vegetative biomass production above 25 mm from the soil surface, number of vegetative tillers, number of flowering tillers and number of seeds. Leaf and vegetative traits were measured in two growing seasons (2013 and 2014) and flower and seed traits were measured in three growing seasons (2013, 2014 and 2015). Table 3.1 gives detailed methods for trait measurement and the timing of measurements. Nineteen of the plants initially planted in the heritability experiment were excluded in the final analysis; justification for their exclusion can be found in Appendix 3, Section A3.1. No trait was missing more than 11 data points, and no phenotypic data were missing at all from the 2013 dataset; thus, the trait data were 99.5% complete.

Table 3.1 Methods for measuring trait data and details of when measurements were taken.

Trait	Method	When
Leaf length (mm)	Length of longest green healthy leaf.	June
Leaf width (mm)	Leaf width was assessed using compound light microscopy. A razor blade was used to take a fine leaf cross section, approximately 10 mm above the base of the longest green healthy leaf. This was placed immediately on a microscope slide with a drop of water and viewed at magnification × 10. Images were taken using a Canon ESO 1000D digital SLR camera mounted on a UNILUX-12 light microscope (Kyowa Optical Co. Ltd). Leaf cross section images were analysed using IMAGEJ (Abramoff, Magalhaes & Ram 2004). Leaf width was measured as twice the distance from the leaf lamina midpoint to the lamina edge (Figure 3.3).	June
Leaf surface area (mm^2)	Surface area of the longest leaf of each plant was calculated as leaf length × leaf width × 1.029. Here, the value 1.029 is a shape constant, which was estimated in a separate trial via a series of leaf-width measurements taken along the entire length of a random sample of *F. ovina* leaves (see Appendix 3, Section A3.2 for full details).	June
Leaf wet mass (mg)	The longest healthy green leaf was weighed, following overnight storage at 100% humidity in a sealed plastic bag at 4 °C, allowing standardisation of leaf turgor (Wilson, Thompson & Hodgson 1999).	July
Leaf dry mass (mg)	The leaf used for measuring wet mass was dried in a paper envelope for 1 week at 55 °C to a constant mass, and then reweighed.	July
Tissue density (%)	Tissue density was calculated as (leaf dry mass/ leaf wet mass) × 100	NA

Specific leaf area (SLA) (mm^2 mg^{-1})	Leaf area divided by leaf dry mass.	NA
Vegetative biomass (mg)	Vegetative biomass was assessed by cutting all the vegetative biomass 25 mm above the soil and dried in a paper envelope for 1 week at 55 °C. The dry biomass was then weighed.	October
Tiller number	A tiller is defined as the collection of blades of grass held within a single sheath (see Figure 977 in Stace 2010). The total number of tillers on the plant was counted. In February 2014 and April 2015, 2 tillers were collected from each plant for use in flow cytometry (see Chapter 4).	September, December, March/April, June
Inflorescence number	A count of the total number of flowering tillers (inflorescences) on an individual plant.	June-July
Seed number	A count of the total number of seeds for all inflorescences on an individual plant.	June-July

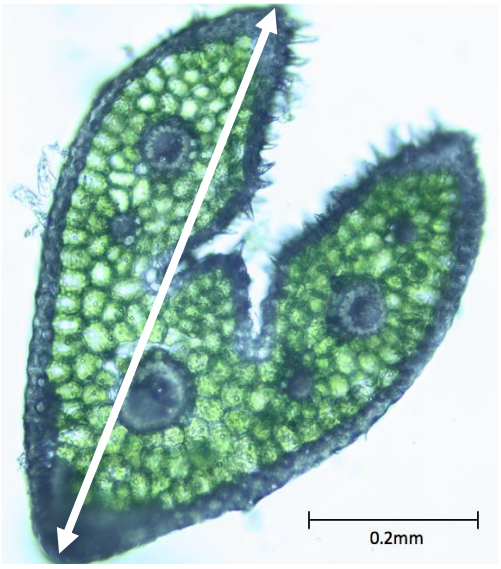

Figure 3.3 Cross sectional view of an *F. ovina* leaf, observed using a compound light microscope. Leaf half-width was measured from the midpoint of the leaf lamina to the edge of the leaf lamina, as indicated by the arrow. Leaf width was taken to be twice this length.

3.3.5 Pedigree

Plants from both the *parent clonal library* and the *offspring clonal library* were genotyped at 9 microsatellite loci, and the genetic data were used to carry out parentage analysis to reconstruct the pedigree (full details in Chapter 2, Section 2.5.3). The identity of the maternal parent of each F1 offspring was known, but the identity of the paternal parent was not. The pedigree was estimated by applying full probability parentage analysis to these microsatellite data, using the R package MASTERBAYES (Hadfield, Richardson & Burke 2006). Summary statistics of the pedigree were calculated using the R package PEDANTICS (Morrissey & Wilson 2010). The pedigree was highly connected, containing 449 maternities, 420 paternities and 177 full sibs (Table 3.2, Figure 3.4).

Table 3.2 Distribution of pairwise relationship types in the *F. ovina* pedigree used here for quantitative genetics analyses, calculated using the R package PEDANTICS (Morrissey & Wilson 2010).

Relationship	**N**
Records	508
Maternities	449
Paternities	420
Full sibs	177
Maternal sibs	1522
Maternal half-sibs	1345
Paternal sibs	2324
Paternal half-sibs	2147

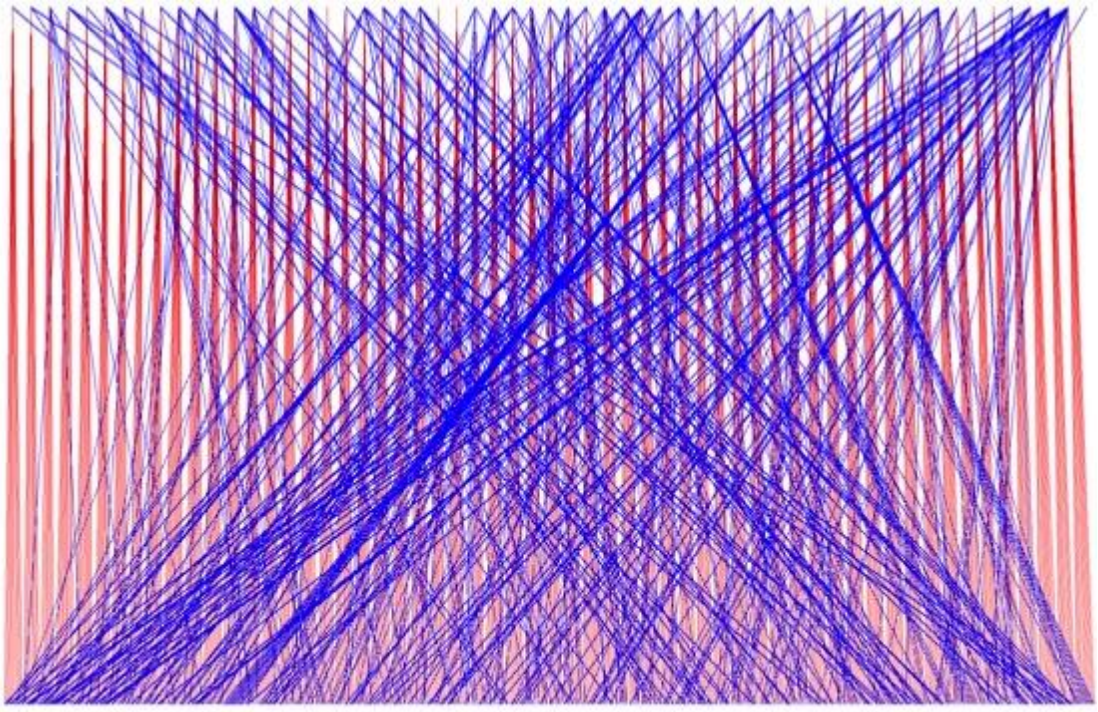

Figure 3.4 Visual depiction of the pedigree for 449 F1 and 59 parent individuals of *F. ovina*, generated using the R package PEDANTICS (Morrissey & Wilson 2010). Parent individuals are shown as nodes on the top edge of the diagram. F1 offspring individuals are shown as nodes on the bottom edge of the diagram. Red lines indicate maternal connections, and blue lines indicate paternal connections. Note that, since *F. ovina* is hermaphroditic, parent individuals at the top of the diagram can be connected to offspring by both red and blue lines.

3.4 Statistical analysis

3.4.1 Quantitative genetic analysis

Quantitative genetic analysis exploits the phenotypic resemblance of relatives to partition total phenotypic variation (V_P), into an additive genetic variance component (V_A), an environmental variance component (V_E), and other non-additive genetic components such at epistasis or dominance effects (Lynch & Walsh 1998; Wilson *et al.* 2010). Narrow sense heritability is defined as the ratio V_A/V_P, and expresses the proportion of variance in phenotype that is attributable to additive genetic variance (Lynch & Walsh 1998). When more than one trait is analysed, the genetic covariance among pairs of traits can also be estimated, which expresses the proportion of variance shared by two traits due to additive genetic causes (Lynch & Walsh 1998).

A variety of statistical methods are available to estimate genetic variance, genetic covariance and heritability. Here, we use two common methods: parent-offspring regression and the animal model. Parent-offspring regression is a long established method for estimating heritability, in which offspring phenotypes are regressed against the mid-parent phenotype, and heritability can then be calculated based on the slope of the regression (Lynch & Walsh 1998). The animal model is a type of mixed-effects model that uses a pedigree to provide information on the relatedness of individuals and to estimate variance components, incorporating information from the full range of relationship types present in the dataset (Kruuk 2004; Wilson *et al.* 2010). The animal model and parent-offspring regression have different advantages and limitations (de Villemereuil, Gimenez & Doligez 2013). The

animal model is highly flexible, able to incorporate unknown parentages, and allows the specification of random effects to account for shared environmental effects, such as *maternal effects* (Kruuk 2004; de Villemereuil, Gimenez & Doligez 2013). Another advantage of the animal model are that they can be fitted as multi-response models, i.e. with multiple traits fitted as the response, which allows the estimation of genetic variance and covariance within and among different traits. However, the animal model is highly computationally intensive. Parent-offspring regression is conceptually simpler than the animal model, and easier to implement, but is unable to incorporate missing data or random effects (Lynch & Walsh 1998; de Villemereuil, Gimenez & Doligez 2013).

Phenotypic covariance among relatives arises partly from additive gene effects and a shared ancestry, but can also be caused by a shared environment. Components of a shared environment must be accounted for appropriately in models in order to derive the best estimates of genetic parameters, but may also be of biological interest in their own right (Kruuk & Hadfield 2007). Maternal effects are a particularly important factor that can increase phenotypic variance among relatives and, as such, can increase estimates of additive genetic variance if not modelled appropriately (Clément *et al.* 2001; Kruuk & Hadfield 2007). Maternal effects are defined as the effect of maternal traits on an offspring's phenotype that are not due to the direct inheritance of genes from the mother (Mousseau & Fox 1998; McAdam, Garant & Wilson 2014). Maternal effects can be partitioned further into the product of the mothers' genotype, *maternal genetic effects*, and the impacts of the environment on the mother, *maternal environmental effects* (Kruuk & Hadfield 2007; McAdam, Garant & Wilson 2014). However, the separate estimation of maternal

genetic and maternal environmental effects requires very large sample sizes and pedigrees of 3 or more generations (Holand & Steinsland 2016).

The reliability of heritability estimates is also influenced by the data structure, the 'connectedness' of the pedigree, and the reliability of the pedigree (Clément *et al.* 2001; Morrissey *et al.* 2007; Wilson *et al.* 2010). Large pedigrees with few connected individuals will have less power to estimate heritability than smaller, highly connected pedigrees (Clément *et al.* 2001; Wilson & Schiff 2010). Molecular methods of pedigree estimation are always associated with some degree of error. In general, misassigned parentages will reduce heritability estimates (Kruuk 2004; Morrissey *et al.* 2007). The effect of pedigree errors on the estimation of maternal effects and genetic architecture is less well understood. However Morrissey *et al.* (2007) found that estimates of genetic architecture were relatively unchanged in simple models (containing only additive genetic effects and residual errors), but biases became larger with more complex models (specifically those also including maternal effects).

Here we estimate heritability and genetic architecture in a population of *F. ovina* collected from BCCIL, using the animal model and parent-offspring regression methods.

3.4.2 The animal model

3.4.2.1 The dataset

The dataset used in this analysis comprised phenotypic trait data for 449 offspring and 55 parent plants, and an associated pedigree that contained the 59

parent plants (lacking parentage information) and the 449 offspring plants with their respective parentage information. Two multi-response animal models were fitted to the data, using the R package MCMCGLMM (Hadfield 2010). All analyses were conducted in R unless stated otherwise (R Development Core Team 2008). First, we fitted a "naïve model" that incorporated additive genetic effects and residual errors, but excluded maternal effects. Second, we fitted a "maternal effects model" that included, in addition, maternal identity as a random effect, to quantify maternal effects and their impact on other parameter estimates. A separate multi-response animal model was carried out for the trait data for each year (2013 and 2014). For each year's data the model contained the following leaf traits: leaf length, leaf width, leaf surface area, leaf wet mass, leaf dry mass, tissue density, SLA, tiller number in September, vegetative biomass collected above 25 mm. Flowering and seed traits were also included in each model as the sum value for each plant across the three years of data collection (2013, 2014 and 2015). Although not ideal, this summed measure for flowering and seed traits was used because the number of plants that flowered in any given year was small. Using the summed measure prevented the dataset from having to be substantially reduced in any given year. Our traits included continuously distributed and count variables; each trait included in the analysis was modelled with an appropriate error distribution, preceded by data transformation where necessary (Table 3.3). Heritability estimates were made on the scale of the transformed variable.

Table 3.3 Data transformations and error distributions used to model traits in quantitative genetics analyses. The same transformations and error distributions were used regardless of the year the data were collected in.

Trait acronym	Trait description	Transformation	Error distribution
Len (mm)	Leaf length	Log	Gaussian
Wid (mm)	Leaf width	None applied	Gaussian
SA (mm^2)	Leaf surface area	Square root	Gaussian
Wm (mg)	Leaf wet mass	Square root	Gaussian
Dm (mg)	Leaf dry mass	Square root	Gaussian
TD (%)	Tissue density	Logit	Gaussian
SLA ($mm^2\ mg^{-1}$)	Specific leaf area	Log	Gaussian
Til	Number of tillers in September	Log	Gaussian
Bio (mg)	Biomass above 25 mm	Square root	Gaussian
TFlw	Total number of flowers summed across 2013, 2014 and 2015	None applied	Poisson
TSeed	Total number of seeds summed across 2013, 2014 and 2015	None applied	Poisson

3.4.2.2 The univariate animal model

The animal model for a single trait, following Wilson *et al.* (2010), i.e. a univariate response model, is specified as:

$$y_i = \mu + a_i + e_i$$

where y_i is the phenotype of the i th individual, μ is the average population phenotype, a_i is the effect of the genotype of individual i relative to μ (also referred to as the breeding value for individual i, representing its additive genetic merit), and

e_i is the residual error. Both a_i and e_i are fitted as random effects. When maternal effects are modelled, we have:

$$y_i = \mu + a_i + m_k + e_i$$

where m_k is a set of random effects that describe the additional contribution of mother *k* to her offspring's phenotype (incorporating both maternal genetic effects and maternal environmental effects). Univariate response animal models estimate a single variance component for each set of random effects, describing the quantity of phenotypic variance attributable to a particular source (e.g. additive genetic, V_A, or maternal, V_M).

3.4.2.3 The multi-response animal model

Animal models can also incorporate responses in more than one trait (multi-response animal models). Such models estimate variance components associated with individual traits and covariance components between pairs of traits, allowing the reconstruction of variance-covariance matrices, with dimensions equal to the number of traits. Specifically, these models decompose the phenotypic variance-covariance matrix **P** additively into variance-covariance matrices for additive genetic effects, **G**, maternal effects, **M**, residual error effects, **R**, and potentially, other additional sources of variance (Wilson *et al.* 2010).

3.4.2.4 Estimating heritability from the animal model

The narrow-sense heritability for traits modelled with a Gaussian error distribution was derived from the variance component estimates as

$$h^2 = \frac{V_A}{V_A + V_R}$$

in naïve models (without mother fitted as a random effect), and as

$$h^2 = \frac{V_A}{V_A + V_M + V_R}$$

in models in which maternal identity had been fitted as a random effect, in both cases following Wilson *et al.* (2010). In these equations, V_A represents the estimate of additive genetic variance, V_M is the estimated variance of the maternal effects and V_R is the estimated residual variance.

For traits that were fitted with a Poisson error distribution (number of flowers and number of seeds), we calculated narrow-sense heritability using a modified equation. We followed the guidelines of Nakagawa & Schielzeth (2010) for estimating variance components from non-Gaussian data following additive over-dispersion models (as implemented in MCMCGLMM). For naïve models, narrow-sense heritability was estimated at the original count scale, following Nakagawa & Schielzeth (2010) as

$$E[Y_{ij}]_A = \exp\left(\beta_o + \frac{V_A + V_R}{2}\right)$$

$$h^2_{poisson} = \frac{E[Y_{ij}]_A \cdot (\exp(V_A) - 1)}{E[Y_{ij}]_A \cdot (\exp(V_A + V_R) - 1) + 1}$$

where Y_{ij} is the observed count for the *i* th individual at the *j* th occasion, $E[Y_{ij}]_A$ is the expectation for Y_{ij} in an additive overdispersion model, V_A is the additive genetic variance, V_R is the residual variance, and β_0 is the population intercept. For maternal effects models, narrow-sense heritability was calculated at the original count scale following Carrasco (2010) and Nakagawa & Schielzeth (2010) as

$$E[Y_{ij}]_A = \exp\left(\beta_o + \frac{V_A + V_M + V_R}{2}\right)$$

$$h^2_{poisson} = \frac{E[Y_{ij}]_A \cdot (\exp(V_A) - 1)}{E[Y_{ij}]_A \cdot (\exp(V_A + V_M + V_R) - 1) + 1}$$

where V_M is the variance caused by maternal effects.

3.4.2.5 Estimating genetic correlations from the animal model

The genetic correlation (r_G), which is a measure of phenotypic covariance between a pair of traits attributable to additive genetic effects (Lynch & Walsh 1998; Wilson *et al.* 2010), was estimated from the posterior distribution for the genetic variance-covariance matrix, following Wilson *et al.* (2010) as

$$r_G = \frac{Cov_{Aij}}{\sqrt{(V_{Ai})(V_{Aj})}}$$

where Cov_{Aij} is the additive genetic covariance between two traits *i* and *j*, V_{Ai} is the additive genetic variance in trait *i* and V_{Aj} is the additive genetic variance in trait *j*. All estimates of r_G and their associated 95% CI are reported on the link scale (as opposed to the data scale).

3.4.2.6 Animal model specifications

Animal models were run in MCMCGLMM for 1,300,000 iterations, with a burn in of 300,000 and a thinning interval of 1,000, resulting in a sample size of 1,000 for parameter estimation. We used an inverse gamma prior distribution for random effects, where $V = 1$ and $nu = 0.002$. Blocking factors describing an individual plant's spatial location (column and row) within the experiment were fitted as centred fixed effects. Autocorrelation (non-independence) between successive samples in the MCMC chain (i.e. at lag 1) was assessed using the *autocorr* function for parameters V_A, V_M and V_R (results given in Appendix 3, Section A3.3, Table A3.1).

We estimated *95% credible intervals* (95% CI) for each parameter via its posterior distribution, using the *HPDinterval* function in MCMCGLMM. The 95% CI represents the range within which we expect the true parameter value to be located with a probability of 0.95. Parameter estimates were considered statistically significant when their credible intervals did not include 0.

3.4.3 Parent-offspring regression

Separate estimates of heritability were made for each trait using parent-offspring regression analysis. These estimates, derived using an extensively-used and

widely-understood method, provide a useful point of comparison for estimates from the more complex animal models. Unlike the animal model, parent-offspring regression cannot cope with missing data. Thus, all available trait data were used for each analysis, and so sample sizes for each analysis differed slightly.

First, full-sib family groups were identified within the pedigree, i.e. unique maternal × paternal parent combinations that yielded one or more offspring with trait observations. The number of families included in each parent-offspring regression analysis ranged from 248 to 267. Family size ranged from a single offspring produced by a unique combination of parents up to 10 full-sib offspring, as summarised in Appendix 3, Section A3.4, Table A3.2.

Regression models were fitted using an iterative weighted least-squares approach, to account for families of different sizes (Lynch & Walsh 1998). Trait data were transformed following Table 3.3. Traits with a Gaussian error distribution were fitted in linear regression models; data with a Poisson error distribution were analysed using a generalised linear model, with a Poisson family. The weighted slope coefficient for each parent-offspring regression model was derived as follows. First, the least-squares regression slope, $b_{o\bar{p}}$, was estimated with no weighting applied. For mid-parent regression, $b_{o\bar{p}}$ is a direct estimate of heritability. Each trait was modelled using the equation

$$z_{oi} = \alpha + \beta_{o\bar{p}} \left(\frac{z_{mi} + z_{pi}}{2}\right) + e_i$$

where z_{oi} is the offspring phenotype for the *i* th family, α is the intercept, $\beta_{o\bar{p}}$ is the regression coefficient, z_{mi} is the phenotype of the maternal parent, z_{pi} is the

phenotype of the paternal parent, and e_i is the residual deviation from the regression.

Next a weighting factor was calculated for each family. The weighting for the *i*th family was calculated as

$$w_i = \frac{n_i}{n_i(t-B)+(1-t)}$$

where n_i is the family size, $t = \frac{\sigma_\alpha^2}{\sigma_\alpha^2 + \sigma_\varepsilon^2}$, the intra-class correlation, σ_α^2 is the variance within family, σ_ε^2 is the variance among families, and $B = \frac{b_{o\bar{p}}^2}{2}$.

Finally, the weighting was applied to the regression, and the weighted least squares regression coefficient, *b*, was calculated as

$$b = \frac{\sum_{i=1}^{N} w_i\,(\bar{z}_{oi}-\bar{z}_o)(z_{\bar{p}i}-\bar{z}_p)}{\sum_{i=1}^{N} w_i(z_{\bar{p}i}-\bar{z}_p)^2}$$

where w_i is the weight for the *i* th family, $\bar{z}_{oi}$ is the mean phenotype of the offspring in the *i* th family, $z_{\bar{p}i}$ is the mid phenotype of the parents for the *i* th family, $\bar{z}_o = \frac{\sum_{i=1}^{N} w_i \bar{z}_{oi}}{\sum_{i=1}^{N} w_i}$ is the weighted mean phenotype for the offspring, and $\bar{z}_p = \frac{\sum_{i=1}^{N} w_i z_{\bar{p}i}}{\sum_{i=1}^{N} w_i}$ is the weighted mean phenotype for the parents.

For each model, the estimate of *B* generated by the un-weighted regression was compared with the estimate obtained from the weighted regression. If the value from the un-weighted regression and the value from the weighted regression were equal (on rounding to three decimal places) then calculation stopped. However, if

the values differed then a new estimate of *B* was calculated from the weighted model and used to generate new weightings. A third regression was subsequently carried out and the entire process was repeated until estimates of *B* were sufficiently similar. For mid-parent regression, $b_{o\bar{p}} = \sqrt{2B}$ is a direct estimate of heritability.

Confidence intervals for the resulting heritability estimates were calculated following Lynch & Walsh (1998) as

$$CI = 2\sqrt{\frac{Var\ (z_o)}{\sum_{i=1}^{N} w_i(z_{\bar{p}i} - \bar{z}_p)^2}}$$

where $Var\ (z_o)$ is the variance in the offspring (other variables are as defined above).

3.4.4 Evolutionary responses to climatic selection at BCCIL

Pedigree information for F1 *F. ovina* individuals included in the common environment experiment defined their *ancestral climatic environment* as either *pure drought ancestry* (both parents from drought plots), *hybrid ancestry* (one parent from each of drought and control) or *pure control ancestry* (both parents from control plots) at BCCIL Table 3.4).

Table 3.4 The ancestral climate assignments of the F1 progeny array

Mother		**Father**	**Offspring**	**N**
Control	×	Control	Pure control ancestry	135
Control	×	Drought	Hybrid ancestry	205
Drought	×	Control		
Drought	×	Drought	Pure drought ancestry	83

If climate treatments at BCCIL have driven evolutionary change in *F. ovina* traits, then F1 plants should be phenotypically differentiated based on their ancestral environment at BCCIL. We used Generalised Linear Mixed-effect Models (GLMMs) to test for this effect, fitted using MCMCGLMM (Hadfield 2010). The phenotypic data used in these analyses were restricted to F1 offspring individuals with complete parentage information, and individuals with missing phenotypic data were excluded on a trait-by-trait basis (minimum sample size = 414). Each trait was modelled according to the transformations and error distributions specified in Table 3.3. The ancestral climate of the F1 *F. ovina* individuals was fitted as a fixed effect with three levels: pure drought ancestry (both parents were from drought plots at BCCIL), pure control ancestry (both parents were from control plots at BCCIL) and hybrid ancestry (one parent was from each of the drought and control treatments at BCCIL). Column and row variables describing an individual plant's spatial location within the experiment were also fitted as fixed effects. Contrasts were set to centre these effects, since they were not of primary interest, to allow estimation of the remaining fixed effects parameters for a notional "average" column and row. The maternal parent and paternal parent of each F1 individual were fitted as random effects. The prior distribution for these variance components was a non-informative uniform improper prior distribution on the standard deviation of the random effects ($V = 1.0 \times 10^{-16}$, $nu = -1$) as recommended by Gelman (2006). Each model was run for 1,300,000 iterations, with a burn in of 300,000 and a thinning interval of 1,000, resulting in a sample size of 1,000 (it was necessary to adjust these parameters for the analysis of total flower number). Each model was run three times with over-dispersed chain starting values using the MCMCGLMM argument *start=list(QUASI=FALSE).* Consistency

in model convergence to a common stationary distribution was assessed via the Gelman-Rubin diagnostic (Gelman & Rubin 1992). We also checked for autocorrelation between successive samples of the MCMC chain for fixed and random effects parameters using the function *autocorr*, to determine their level of independence. Autocorrelation values below 0.15 were required for the model to be accepted, following Hadfield (2012). We report "*95% credible intervals"* which is the range within which we expect, with a probability of 0.95, the true parameter value to be located. Credible intervals were calculated using the *HPDinterval* function in MCMCGLMM. Reported *pMCMC* values are for the comparison between plants from pure control ancestry and pure drought ancestry.

3.5 Results

3.5.1 Summary of plant phenotypes

Plant size in the common environment experiment ranged from a single tiller to 45 tillers. No plant produced more than three flowering tillers in any single year of observation (Appendix 3, Section A3.5, Table A3.3). Individual plants also varied greatly in the total number of flowering tillers and seeds that they produced over three years (Figure 3.5 **A**, **B** & **C**; Table 3.5). The total number of seeds produced by a plant increased with increased number of flowers produced (Figure 3.5 **A**). The total number of seeds produced by a plant also varied depending on the year(s) in which the plant produced flowers (Figure 3.5 **B**). Among plants that only produced flowers in a single year, the number of seeds produced decreased from 2013 to 2015. There

was a trade-off between the number of tillers that a plant had and the number of seeds that it produced (Figure 3.5 **C**): plants could produce many tillers and few seeds, or few tillers and many seeds, or few tillers and few seeds; however, no individual could produce many tillers and many seeds.

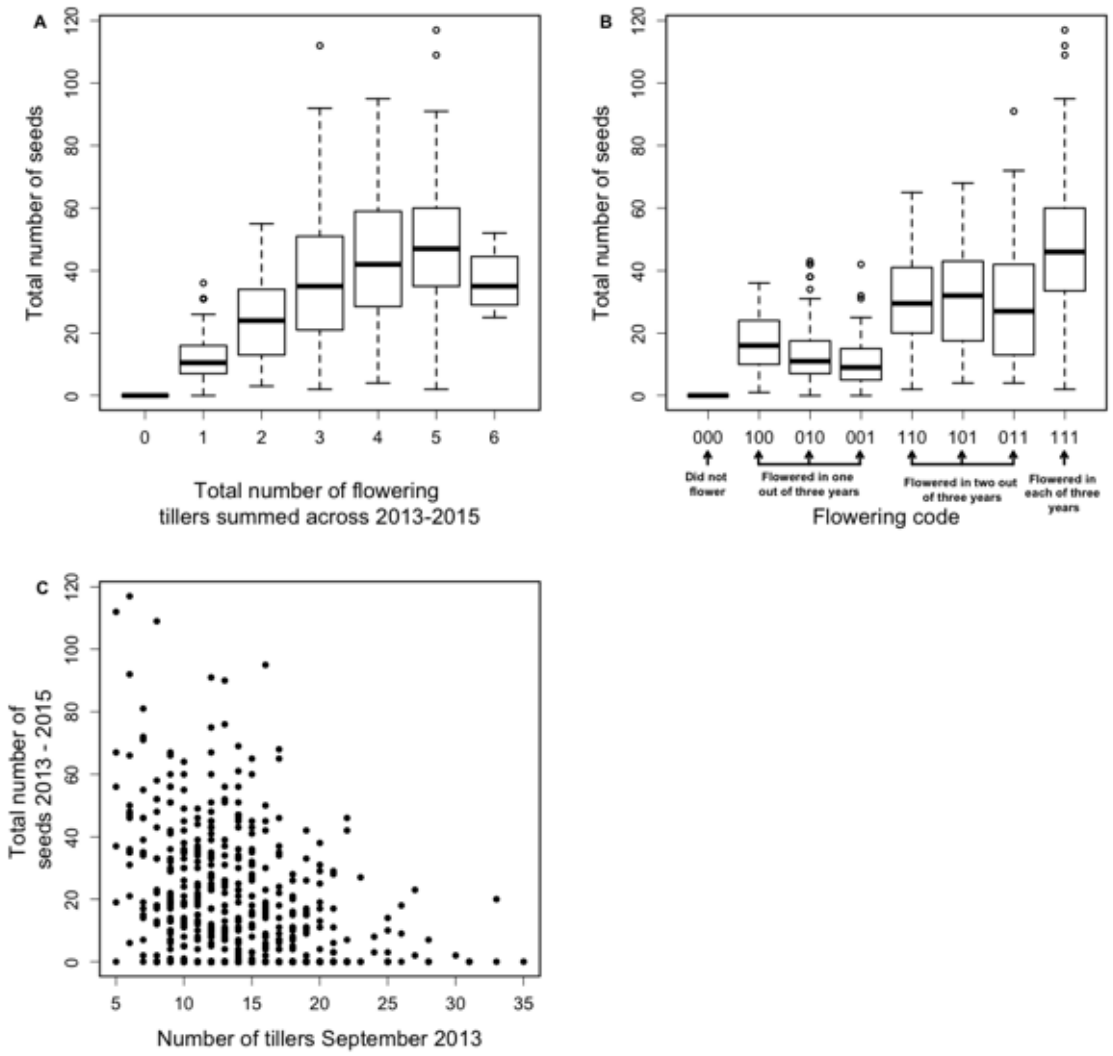

Figure 3.5 Variation in the flowering and seed production of F1 *F. ovina* plants during three years' growth in a common environment experiment (2013–2015). **A)** Total number of seeds produced against total number of flowering tillers produced. **B)** Total number of seeds produced in plants making different numbers of flowering attempts. The flowering code shown in (B) is a three digit binary code, 0 = the plant did not flower, 1 = the plant flowered, with each digit corresponding to flowering seasons in 2013, 2014 and 2015, respectively. Boxes shown in boxplots delimit the first and third quartiles of the data; whiskers represent ± 1.5 × the interquartile range, with points lying outside these whiskers representing outliers. **C)** Plant tiller count in September 2013 versus total number of seeds produced over three years. Each point represents a single plant.

Table 3.5 Phenotypic mean values, standard errors and the range of trait values measured on *F. ovina*. The data presented for total number of flowering tillers and total number of seeds were calculated as the sum of 2013, 2014 and 2015. There were 131 plants that never flowered during the course of the experiment and these are included in the averages. Values in brackets are if the plants that did not produce flowers in any year are excluded.

Trait	**Mean**		**S.E**		**Range**		**N**	
	2013	***2014***	***2013***	***2014***	***2013***	***2014***	***2013***	***2014***
Len (mm)	39.687	45.410	0.448	0.525	18 – 75	15 – 84	504	500
Wid (mm)	1.112	1.060	0.006	0.006	0.698 – 1.540	0.650 – 1.570	504	500
SA (mm^2)	45.582	49.924	0.600	0.690	18.883 – 97.662	10.904 – 105.572	504	500
Wm (mg)	6.412	6.695	0.109	0.121	1.870 – 19.120	0.890 – 15.200	504	494
Dm (mg)	2.195	2.982	0.043	0.056	0.460 – 8.060	0.100 – 7.000	504	499
TD (%)	33.951	44.467	0.198	0.320	17.230 – 44.580	10.526 – 93.333	504	493
SLA ($mm^2\ mg^{-1}$)	22.297	18.297	0.234	0.351	11.009 – 45.527	9.842 – 126.652	504	499
Til	14	11	0.226	0.190	5 – 35	3 – 32	504	504
Bio (mg)	17.504	15.825	0.482	0.446	0.280 – 73.50	2.070 – 85.700	504	504
TFlw‡	1.710 (2.311)		0.066 (0.065)		0 – 6 (1 – 6)		504 (373)	
TSeed‡	19.497 (26.475)		0.945 (1.069)		0 – 117 (0 – 117)		497 (366)	

Len = leaf length; Wid = leaf width; SA = leaf surface area; Wm = Wet mass; Dm = Dry mass; TD = tissue density; SLA = specific leaf area; Til = number of tillers in September; Bio = biomass above 25 mm; TFlw = total number of flowers summed across 3 years; TSeed = total number of seeds summed across 3 years. ‡ These are the values summed across three years, 2013, 2014 and 2015.

3.5.2 Heritability

Parent-offspring regression identified significant narrow-sense heritability (h^2) in all traits except leaf width, leaf wet mass and tissue density in 2014 (Table 3.6). Estimates of heritability from the parent-offspring regression ranged from $h^2 = 0.010$, for number of seeds, to $h^2 = 0.304$ for number of tillers (Table 3.6). All heritability values calculated by the naïve and maternal effects animal model were significantly greater than zero (Table 3.6). Heritability estimates calculated with the naïve animal model ranged from $h^2 = 0.042$ for number of seeds, to $h^2 = 0.554$ for leaf width. Heritability estimates calculated with the maternal effects animal model ranged from $h^2 = 0.014$ for number of seeds, to $h^2 = 0.300$ for number of tillers. A breakdown of the variance components used to estimate heritability is provided in Appendix 3, Section A3.6, Table A3.4.

The maternal component had a large influence on estimates of heritability, which were considerably lower when a maternal variance component was incorporated into the animal model. For example, for the trait leaf width, the estimate of narrow-sense heritability more than halved once the maternal effects were included ($h^2 = 0.550$, naïve model and $h^2 = 0.210$, maternal effects model, 2013 data). On average the maternal variance component accounted for 41.1% of the total phenotypic variance, ranging from 13.5% to 60.5%, depending on trait and year of observation.

Table 3.6 Estimates of narrow sense heritability (h^2) calculated from the animal model and parent-offspring regression methods. CI† = 95% credible interval. CI^ = 95% confidence interval. For the animal model, heritability was considered to be significantly greater than zero when its corresponding credible interval (range from lower to upper CI) did not include 0. For parent-offspring regression significance levels are given as follows: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$, p values drawn from the model output.

		Animal model						*Parent-offspring regression*		
		Maternal effects model			*Naïve model*					
Trait		***h²***	***Lower CI†***	***Upper CI†***	***h²***	***Lower CI†***	***Upper CI†***	***h²***	***Lower CI^***	***Upper CI^***
Len	2013	0.232	0.166	0.286	0.457	0.402	0.528	0.239***	0.096	0.381
	2014	0.226	0.162	0.283	0.458	0.401	0.533	0.180**	0.058	0.303
Wid	2013	0.210	0.162	0.273	0.550	0.507	0.610	0.186*	0.037	0.335
	2014	0.223	0.162	0.273	0.554	0.492	0.601	0.156	–0.018	0.330
SA	2013	0.279	0.186	0.399	0.324	0.215	0.419	0.207**	0.073	0.342
	2014	0.259	0.161	0.365	0.295	0.196	0.429	0.161*	0.027	0.295
Wm	2013	0.269	0.185	0.338	0.388	0.326	0.498	0.302***	0.172	0.431
	2014	0.233	0.166	0.319	0.377	0.294	0.486	0.132	–0.004	0.268
Dm	2013	0.231	0.174	0.299	0.434	0.373	0.508	0.259***	0.147	0.370
	2014	0.223	0.177	0.310	0.411	0.346	0.513	0.210***	0.084	0.337
TD	2013	0.221	0.176	0.295	0.529	0.473	0.591	0.139*	0.010	0.269
	2014	0.267	0.207	0.340	0.485	0.431	0.580	–0.002	–0.126	0.121
SLA	2013	0.221	0.182	0.300	0.514	0.464	0.581	0.249***	0.115	0.384
	2014	0.237	0.177	0.303	0.521	0.444	0.577	0.163*	0.030	0.297
Til	2013	0.294	0.204	0.342	0.502	0.432	0.583	0.304***	0.173	0.434
	2014	0.287	0.213	0.346	0.515	0.440	0.593	0.232**	0.077	0.388
Bio	2013	0.300	0.213	0.443	0.295	0.212	0.383	0.247**	0.091	0.403
	2014	0.290	0.179	0.401	0.387	0.260	0.508	0.244**	0.094	0.393
TFlw‡		0.083	0.037	0.174	0.163	0.083	0.285	0.150***	0.007	0.293
TSeed‡		0.014	0.004	0.053	0.042	0.012	0.105	0.010***	–0.116	0.136

Len = leaf length; Wid = leaf width; SA = leaf surface area; Wm = wet mass; Dm = Dry mass; TD = tissue density; SLA = specific leaf area; Til = number of tillers in September; Bio = biomass above 25 mm; TFlw = total number of flowers summed across 3 years; TSeed = total number of seeds summed across 3 years. ‡ These are the values summed across three years, 2013, 2014 and 2015. Heritability estimates for TFlw and TSeed are taken from multivariate models for 2013 data.

3.5.3 Genetic architecture

The genetic correlations among traits were broadly similar between the naïve model and the maternal effects model for 2013 and 2014 (Table 3.7, 3.8, 3.9, and 3.10). In the 2013 growing season, 12 positive and 6 negative genetic correlations were significantly supported in both the naïve and maternal effects model (out of 55 genetic correlations calculated in each model). Three pairs of genetic correlations were differently supported by the naïve and maternal effects model (leaf dry mass with tissue density, leaf length with tiller number, and tiller number with total number of seeds). However, in each of these cases the direction and magnitude were similar to those in the alternative model. In the 2014 growing season, 11 positive and 3 negative genetic correlations were significantly supported in both the naïve and maternal effects model. Two pairs of genetic correlations were differently supported by the naïve and maternal effects model (leaf length with biomass, leaf dry mass with tissue density). Again, in these cases the correlations were in the same direction and at a similar magnitude to those in the alternative model. Statistically supported genetic correlations ranged from r_G = –0.299 (specific leaf area and leaf dry mass, naïve model, 2014), to r_G = 0.839 (total seed number and total flower number, naïve model, 2013). Further comparisons focus solely on the maternal effects models.

Genetic correlations were, on the whole, consistent between years of observation. The genetic correlation between leaf dry mass and tissue density was significantly greater than zero in 2014 but not in 2013, though the values were very similar in magnitude in each year (2013, r_G = 0.167 and 2014, r_G = 0.180; Table 3.9 & 3.10). Genetic correlations between leaf surface area and tiller number, wet mass

and tiller number, leaf length and vegetative biomass, specific leaf area and biomass, and number of tillers and total number of seeds decreased in strength and lost statistical significance from 2013 to 2014. For example, in 2013 the genetic correlation between leaf surface area and tiller number was r_G = –0.260, compared to r_G = –0.100 in 2014 (Table 3.9 & 3.10), and in 2013 the genetic correlation between number of tillers and total number of seeds was r_G = –0.205, compared to r_G = –0.149 in 2014 (Table 3.9 & 3.10).

3.5.4 Evolutionary responses to climatic selection at BCCIL

The ancestral climatic environment at BCCIL was not a significant predictor of any of the traits values analysed in either 2013 or 2014, Table 3.11.

Table 3.7 Genetic correlations (r_G) among 11 traits in *F. ovina* from the naïve model, 2013 data. Brackets show the 95% credible intervals. Bold values indicate statistically supported correlations, where significance is defined as credible intervals that do not include 0.

2013	Wid	SA	Wm	Dm	TD	SLA	Til	Bio	TFlw‡	TSeed‡
Len	–0.034 (-0.134 – 0.122)	**0.411** (0.254 – 0.525)	**0.259** (0.125 – 0.421)	**0.184** (0.075 – 0.348)	0.063 (-0.070 – 0.195)	–0.058 (-0.210 – 0.071)	**–0.193** (-0.297 – -0.013)	**0.205** (0.083 – 0.385)	0.038 (-0.155 – 0.237)	0.075 (-0.167 – 0.234)
Wid	----------	**0.143** (0.060 – 0.342)	0.102 (-0.029 – 0.244)	0.029 (-0.057 – 0.188)	–0.039 (-0.155 – 0.104)	0.057 (-0.121 – 0.148)	–0.055 (-0.161 – 0.117)	–0.069 (-0.152 – 0.133)	0.069 (-0.120 – 0.180)	0.032 (-0.107 – 0.198)
SA	----------	----------	**0.594** (0.433 – 0.694)	**0.429** (0.276 – 0.555)	–0.011 (-0.143 – 0.170)	–0.069 (-0.240 – 0.093)	**–0.238** (-0.426 – -0.084)	**0.383** (0.128 – 0.536)	0.142 (-0.194 – 0.364)	0.156 (-0.165 – 0.422)
Wm	----------	----------	----------	**0.394** (0.290 – 0.558)	–0.018 (-0.129 – 0.166)	**–0.219** (-0.382 – -0.094)	**–0.197** (-0.355 – -0.016)	**0.293** (0.153 – 0.509)	0.067 (-0.202 – 0.283)	0.124 (-0.198 – 0.304)
Dm	----------	----------	----------	----------	**0.121** (0.019 – 0.282)	**–0.279** (-0.394 – -0.124)	–0.109 (-0.264 – 0.051)	**0.301** (0.123 – 0.450)	–0.031 (-0.227 – 0.206)	0.024 (-0.239 – 0.216)
TD	----------	----------	----------	----------	----------	**–0.215** (-0.316 – -0.061)	0.041 (-0.094 – 0.182)	0.155 (-0.028 – 0.296)	–0.134 (-0.236 – 0.079)	–0.089 (-0.238 – 0.085)
SLA	----------	----------	----------	----------	----------	----------	–0.022 (-0.154 – 0.134)	**–0.180** (-0.359 – -0.044)	0.063 (-0.089 – 0.240)	0.058 (-0.107 – 0.245)
Til	----------	----------	----------	----------	----------	----------	----------	–0.083 (-0.286 – 0.076)	–0.202 (-0.357 – 0.029)	–0.179 (-0.372 – 0.017)
Bio	----------	----------	----------	----------	----------	----------	----------	----------	0.007 (-0.296 – 0.279)	–0.035 (-0.308 – 0.273)
TFlw‡	----------	----------	----------	----------	----------	----------	----------	----------	----------	**0.839** (0.662 – 0.899)

Len = leaf length; Wid = leaf width; SA = leaf surface area; Wm= wet mass (mg); Dm = dry mass (mg); TD = tissue density; SLA = specific leaf area; Til = number of tillers in September; Bio = biomass above 25 mm; TFlw = total number of flowers summed across 3 years; TSeed = total number of seeds summed across 3 years. ‡ These are the values summed across three years, 2013, 2014 and 2015.

Table 3.8 Genetic correlations (r_G) among 11 traits in *F. ovina* from the naïve model, 2014 data. Brackets show the 95% credible intervals. Bold values indicate statistically supported correlations, where significance is defined as credible intervals that do not include 0.

2014	Wid	SA	Wm	Dm	TD	SLA	Til	Bio	TFlw‡	TSeed‡
Len	0.048 (-0.125 – 0.156)	**0.463** (0.268 – 0.567)	**0.312** (0.185 – 0.486)	**0.305** (0.142 – 0.447)	0.078 (-0.087 – 0.215)	–0.141 (-0.262 – 0.021)	–0.059 (-0.225 – 0.072)	**0.166** (0.028 – 0.384)	–0.136 (-0.300 – 0.106)	–0.057 (-0.287 – 0.141)
Wid	----------	**0.230** (0.059 – 0.345)	0.131 (-0.033 – 0.265)	0.121 (-0.064 – 0.225)	–0.023 (-0.132 – 0.146)	–0.012 (-0.126 – 0.141)	0.034 (-0.117 – 0.137)	0.025 (-0.112 – 0.185)	–0.041 (-0.193 – 0.114)	–0.064 (-0.202 – 0.118)
SA	----------	----------	**0.622** (0.479 – 0.751)	**0.535** (0.368 – 0.651)	0.120 (-0.078 – 0.301)	–0.175 (-0.330 – 0.004)	–0.163 (-0.282 – 0.079)	**0.309** (0.061 – 0.566)	–0.144 (-0.462 – 0.130)	–0.150 (-0.499 – 0.153)
Wm	----------	----------	----------	**0.468** (0.349 – 0.627)	–0.023 (-0.184 – 0.165)	**–0.273** (-0.406 – -0.108)	–0.084 (-0.249 – 0.085)	**0.313** (0.065 – 0.498)	–0.139 (-0.369 – 0.131)	–0.129 (-0.367 – 0.172)
Dm	----------	----------	----------	----------	0.169 (-0.010 – 0.297)	**–0.299** (-0.422 – -0.151)	–0.062 (-0.201 – 0.119)	**0.232** (0.024 – 0.432)	–0.128 (-0.345 – 0.100)	–0.129 (-0.351 – 0.116)
TD	----------	----------	----------	----------	----------	**–0.206** (-0.375 – -0.103)	0.031 (-0.134 – 0.190)	0.027 (-0.153 – 0.216)	–0.043 (-0.264 – 0.134)	–0.073 (-0.273 – 0.115)
SLA	----------	----------	----------	----------	----------	----------	–0.035 (-0.182 – 0.096)	–0.136 (-0.292 – 0.063)	0.084 (-0.097 – 0.265)	0.083 (-0.115 – 0.254)
Til	----------	----------	----------	----------	----------	----------	----------	0.099 (-0.083 – 0.282)	–0.078 (-0.240 – 0.164)	–0.063 (-0.281 – 0.135)
Bio	----------	----------	----------	----------	----------	----------	----------	----------	–0.152 (-0.434 – 0.134)	–0.217 (-0.448 – 0.133)
TFlw‡	----------	----------	----------	----------	----------	----------	----------	----------	----------	**0.830** (0.671 – 0.890)

Len = leaf length; Wid = leaf width; SA = leaf surface area; Wm= wet mass (mg); Dm = dry mass (mg); TD = tissue density; SLA = specific leaf area; Til = number of tillers in September; Bio = biomass above 25 mm; TFlw = total number of flowers summed across 3 years; TSeed = total number of seeds summed across 3 years. ‡ These are the values summed across three years, 2013, 2014 and 2015.

Table 3.9 Genetic correlations (r_G) among 11 traits in *F. ovina* from the maternal effects model, 2013 data. Brackets show the 95% credible intervals. Bold values indicate statistically supported correlations, where significance is defined as credible intervals that do not include 0.

2013	Wid	SA	Wm	Dm	TD	SLA	Til	Bio	TFlw‡	TSeed‡
Len	–0.010 (-0.158 – 0.133)	**0.372** (0.248 – 0.552)	**0.301** (0.118 – 0.435)	**0.217** (0.080 – 0.374)	0.047 (-0.087 – 0.183)	–0.054 (-0.216 – 0.070)	–0.147 (-0.296 – 0.006)	**0.289** (0.090 – 0.434)	0.049 (-0.134 – 0.279)	0.127 (-0.128 – 0.291)
Wid	----------	**0.160** (0.028 – 0.323)	0.105 (-0.038 – 0.249)	0.038 (-0.082 – 0.200)	–0.032 (-0.179 – 0.105)	0.016 (-0.137 – 0.149)	–0.005 (-0.175 – 0.111)	0.033 (-0.162 – 0.145)	0.072 (-0.108 – 0.205)	0.071 (-0.135 – 0.198)
SA	----------	----------	**0.587** (0.440 – 0.721)	**0.459** (0.289 – 0.608)	0.019 (-0.117 – 0.217)	–0.101 (-0.262 – 0.089)	**–0.260** (-0.451 – -0.079)	**0.431** (0.192 – 0.648)	0.210 (-0.132 – 0.491)	0.251 (-0.099 – 0.555)
Wm	----------	----------	----------	**0.451** (0.293 – 0.575)	0.036 (-0.126 – 0.189)	**–0.225** (-0.397 – -0.088)	**–0.198** (-0.350 – -0.019)	**0.399** (0.182 – 0.584)	0.101 (-0.171 – 0.360)	0.142 (-0.173 – 0.406)
Dm	----------	----------	----------	----------	0.167 (-0.010 – 0.283)	**–0.259** (-0.401 – -0.126)	–0.077 (-0.298 – 0.031)	**0.356** (0.161 – 0.512)	0.079 (-0.183 – 0.275)	0.042 (-0.201 – 0.295)
TD	----------	----------	----------	----------	----------	**–0.218** (-0.334 – -0.062)	0.025 (-0.097 – 0.173)	0.152 (-0.024 – 0.298)	–0.032 (-0.234 – 0.123)	–0.010 (-0.256 – 0.111)
SLA	----------	----------	----------	----------	----------	----------	0.007 (-0.158 – 0.134)	**–0.201** (-0.363 – -0.039)	0.052 (-0.144 – 0.231)	0.052 (-0.134 – 0.247)
Til	----------	----------	----------	----------	----------	----------	----------	–0.109 (-0.331 – 0.061)	–0.164 (-0.401 – 0.030)	**–0.205** (-0.427 – -0.004)
Bio	----------	----------	----------	----------	----------	----------	----------	----------	0.081 (-0.245 – 0.375)	0.105 (-0.216 – 0.429)
TFlw‡	----------	----------	----------	----------	----------	----------	----------	----------	----------	**0.780** (0.572-0.883)

Len = leaf length; Wid = leaf width; SA = leaf surface area; Wm= wet mass (mg); Dm = dry mass (mg); TD = tissue density; SLA = specific leaf area; Til = number of tillers in September; Bio = biomass above 25 mm; TFlw = total number of flowers summed across 3 years; TSeed = total number of seeds summed across 3 years. ‡ These are the values summed across three years, 2013, 2014 and 2015.

Table 3.10 Genetic correlations (r_G) among 11 traits in *F. ovina* from the maternal effects model, 2014 data. Brackets show the 95% credible intervals. Bold values indicate statistically supported correlations, where significance is defined as credible intervals that do not include 0.

2014	Wid	SA	Wm	Dm	TD	SLA	Til	Bio	TFlw‡	TSeed‡
Len	–0.009 (-0.136 – 0.150)	**0.432** (0.253 – 0.561)	**0.339** (0.144 – 0.465)	**0.265** (0.124 – 0.440)	0.056 (-0.089 – 0.211)	–0.113 (-0.263 – 0.034)	–0.095 (-0.258 – 0.049)	0.192 (-0.003 – 0.379)	–0.080 (-0.237 – 0.178)	–0.028 (-0.259 – 0.176)
Wid	----------	**0.165** (0.047 – 0.343)	0.069 (-0.061 – 0.238)	0.089 (-0.066 – 0.219)	–0.024 (-0.123 – 0.170)	–0.007 (-0.130 – 0.163)	–0.015 (-0.117 – 0.165)	0.057 (-0.129 – 0.187)	–0.061 (-0.188 – 0.140)	–0.005 (-0.206 – 0.138)
SA	----------	----------	**0.608** (0.429 – 0.722)	**0.497** (0.336 – 0.647)	0.072 (-0.122 – 0.266)	–0.175 (-0.356 – 0.027)	–0.100 (-0.305 – 0.087)	**0.285** (0.063 – 0.601)	–0.080 (-0.434 – 0.222)	–0.098 (-0.430 – 0.276)
Wm	----------	----------	----------	**0.472** (0.301 – 0.592)	–0.019 (-0.181 – 0.165)	**–0.206** (-0.399 – -0.083)	–0.056 (-0.244 – 0.112)	**0.217** (0.025 – 0.492)	–0.023 (-0.326 – 0.217)	0.013 (-0.338 – 0.257)
Dm	----------	----------	----------	----------	**0.180** (0.001 – 0.330)	**–0.268** (-0.427 – -0.138)	–0.023 (-0.213 – 0.129)	**0.212** (0.020 – 0.436)	–0.095 (-0.289 – 0.214)	–0.046 (-0.332 – 0.207)
TD	----------	----------	----------	----------	----------	**–0.199** (-0.370 – -0.080)	0.053 (-0.123 – 0.186)	0.041 (-0.132 – 0.221)	–0.060 (-0.251 – 0.154)	–0.070 (-0.273 – 0.132)
SLA	----------	----------	----------	----------	----------	----------	–0.037 (-0.200 – 0.100)	–0.134 (-0.288 – 0.064)	0.019 (-0.125 – 0.246)	0.066 (-0.157 – 0.250)
Til	----------	----------	----------	----------	----------	----------	----------	0.111 (-0.103 – 0.299)	–0.107 (-0.285 – 0.145)	–0.149 (-0.307 – 0.111)
Bio	----------	----------	----------	----------	----------	----------	----------	----------	–0.158 (-0.432 – 0.161)	–0.129 (-0.440 – 0.192)
TFlw‡	----------	----------	----------	----------	----------	----------	----------	----------	----------	**0.800** (0.572 – 0.877)

Len = leaf length; Wid = leaf width; SA = leaf surface area; Wm= wet mass (mg); Dm = dry mass (mg); TD = tissue density; SLA = specific leaf area; Til = number of tillers in September; Bio = biomass above 25 mm; TFlw = total number of flowers summed across 3 years; TSeed = total number of seeds summed across 3 years. ‡ These are the values summed across three years, 2013, 2014 and 2015.

Table 3.11 Predicted trait means for F1 *F. ovina* plants by ancestral climate treatment at BCCIL. Fitted effects have been back-transformed from the scale of the model linear predictor. CI[†] = 95% credible interval. *pMCMC* values are given for the climate treatment main effect (two levels).

Trait		Pure control	*Lower CI*[†]	*Upper CI*[†]	Pure drought	*Lower CI*[†]	*Upper CI*[†]	*pMCMC*	N
Len (mm)	2013	37.621	35.290	39.372	37.455	34.457	39.892	0.954	423
	2014	42.842	40.370	45.466	43.532	40.108	46.574	0.798	420
Wid (mm)	2013	1.130	1.097	1.168	1.126	1.085	1.172	0.908	423
	2014	1.061	1.025	1.100	1.061	1.009	1.101	0.958	420
SA (mm^2)	2013	44.425	41.317	47.498	44.082	40.897	47.842	0.900	423
	2014	47.307	43.290	51.197	48.816	44.415	53.683	0.668	420
Wm (mg)	2013	6.042	5.449	6.662	5.940	5.234	6.622	0.816	423
	2014	6.258	5.577	6.689	6.307	5.585	7.193	0.892	414
Dm (mg)	2013	2.020	1.800	2.278	1.992	1.739	2.256	0.892	423
	2014	2.743	2.429	3.038	2.762	2.370	3.138	0.974	420
TD (%)	2013	33.462	32.251	34.491	33.345	30.536	36.446	0.916	423
	2014	44.422	42.514	46.416	42.374	40.265	44.750	0.240	414
SLA (mm^2/mg)	2013	22.354	20.878	23.718	22.412	20.836	24.194	0.950	423
	2014	17.624	16.678	18.713	18.707	17.318	19.909	0.280	420
Til	2013	13.283	12.131	14.28	12.788	11.604	13.978	0.578	423
	2014	10.157	9.270	11.146	9.427	8.459	10.552	0.412	423
Bio (mg)	2013	15.288	12.981	17.552	13.931	11.472	16.601	0.458	423
	2014	13.813	11.704	15.844	14.306	11.648	16.626	0.788	423
TFlw[‡]		1.393	1.026	1.807	1.361	0.946	1.821	0.916	420
TSeed[‡]		5.199	2.482	8.908	7.242	2.299	12.556	0.566	414

Len = leaf length; Wid = leaf width; SA = leaf surface area; TD = tissue density; SLA = specific leaf area; Til = number of tillers in September; Bio = biomass above 25 mm; TFlw = total number of flowers summed across 3 years; TSeed = total number of seeds summed across 3 years. [‡] These are the values summed across three years, 2013, 2014 and 2015.

3.6 Discussion

In this chapter we have used quantitative genetic analysis to measure the heritability and genetic architecture of morphological and reproductive traits in a population of *F. ovina* from BCCIL. We found that most traits have moderate levels of heritability, but that maternal effects also explain a substantial proportion of phenotypic variation. Furthermore, we have identified negative genetic correlations between asexual and sexual reproductive traits, specifically number of tillers and number of seeds, suggesting that there are genetically determined trade-offs in life-history strategy in *F. ovina*. Finally, we have not observed phenotypic differentiation between plants of drought or control ancestry at BCCIL when grown in a common environment, which suggests that this population has not shown an evolutionary response to climatic selection.

3.6.1 Heritability

We estimated heritability using three different methods in morphological and reproductive traits in *F. ovina.* Heritability estimates from the animal model (with maternal effects) and parent-offspring regression were consistent with each other. However, estimates calculated using an animal model without maternal effects were consistently much greater than those obtained from the other two methods. This suggests that there is a strong maternal component to phenotypic variance in this population, and justifies the inclusion of maternal effects in the animal model. These maternal effects accounted for between 13.5% and 60.5% of the total phenotypic variance in any given trait. Heritability estimates from 2013

and 2014 data were very similar to each other, suggesting that underlying sources of variance may be comparable through time. Although maternal effects are often expected to be strongest in early-acting life-history traits, other studies have found that maternal effects can persist to influence traits expressed later in the lifecycle (Richardson & Stephenson 1992; Campbell 1997; Thiede 1998; Steets & Ashman 2010).

The estimates of heritability for reproductive traits (total flower number and total number of seeds) were lower than those for morphological traits. This result corresponds with the literature, which has tended to find lower heritability estimates for traits closely linked to fitness compared with other morphological traits (Merilä & Sheldon 1999; Visscher, Hill & Wray 2008). Our heritability estimates are lower in comparison with the findings of other studies focusing on *Festuca* species. The heritability of traits in *F. arundinacea* has been particularly well studied because of its importance as a forage grass. Thomas (1967) estimated narrow-sense heritability in two populations of *F. arundinacea*, with different crossing methods (open pollinated, self-seeded and specific crossing) returning heritability estimates ranging between $h^2 = 0.000 – 0.936$ for number of tillers, and $h^2 = 0.141 – 0.753$ for number of seeds. A more recent estimate supports a high value for the heritability of number of tillers $h^2 = 0.88 \pm 0.61$ (Saxena 2014). The heritability of tiller number has also been estimated in *F. rubra,* and was found to be very high, $h^2 = 0.96 \pm 0.24$ (Rhebergen & Nelissen 1985). Estimates of the heritability of dry matter yield (which is similar to our vegetative biomass measurement) range from $h^2 = 0.34 – 0.46$ (Majidi, Mirlohi & Amini 2009) to $h^2 = 0.58 – 0.60$ (Annicchiarico & Romani 2005), in *F. arundinacea*.

3.6.2 Genetic architecture

We calculated genetic correlations between pairs of traits better to understand how genetic correlations may potentially constrain evolutionary responses to climate change. Further discussion will focus only on the results from the maternal effects model. A number of the statistically significant genetic correlations that we identified were to be expected, because some traits were calculated as functions of others (e.g. leaf length, leaf width and leaf surface area). Other non-derived, but still predictable trait correlations, include positive correlations between aspects of leaf size with plant biomass production, which reflects genetic differences in plant size. Of most interest are the genetic correlations between traits that indicate genetically determined trade-offs in life-history strategy. We found negative genetic correlations between leaf surface area and number of tillers, leaf wet mass and number of tillers, and number of tillers and total number of seeds. Each of these correlations was significantly supported in 2013 but not in 2014, though remaining in the same direction. Genetic architecture is known to depend on the environmental conditions in which traits are expressed: thus, environmental differences between 2013 and 2014 may explain these differences (Sgrò & Hoffmann 2004; Visscher, Hill & Wray 2008).

The negative genetic correlations between leaf surface area and number of tillers, and between leaf wet mass and number of tillers, suggest that plant growth is genetically constrained. Specifically, plants with additive genetic variance for large leaves may have few tillers, and vice versa. This trade-off reflects visible differences in plant phenotypes, with a small-leaved dense tussock-forming phenotype contrasting with a large-leaved dispersed-tiller phenotype (Figure 3.6).

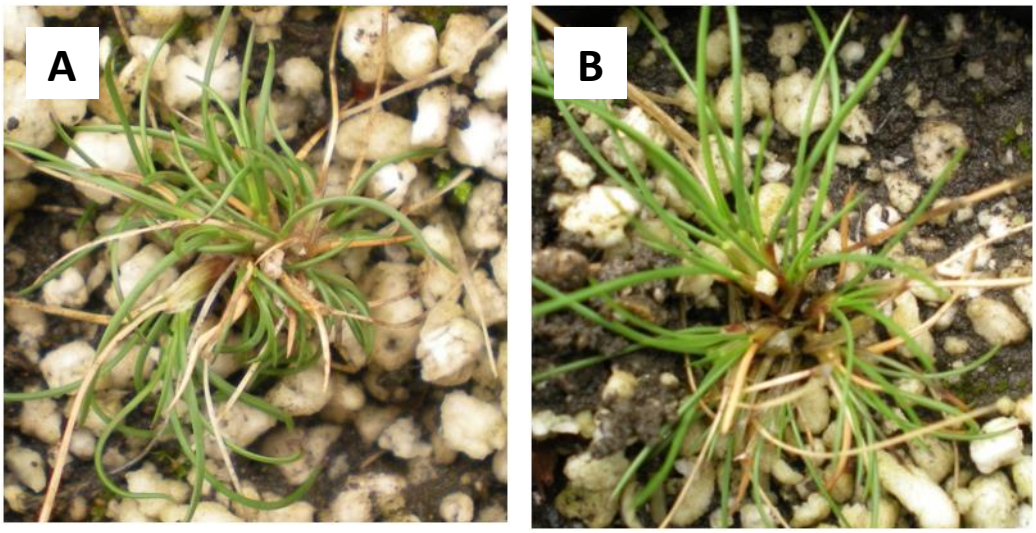

Figure 3.6 *Festuca ovina* phenotypes reflecting the negative genetic correlation between leaf size and number of tillers. **A)** small-leaved dense tussock phenotype and **B)** large leaved dispersed tiller phenotype.

The negative genetic correlation between number of tillers and total number of seeds indicates a trade-off between asexual and sexual reproduction. Negative correlations between sexual and asexual reproduction, or sexual reproduction and vegetative growth, have been well described in life-history trade-off theory (Stearns 1992; Obeso 2002). A genetic basis underlying these trade-offs has been identified experimentally in some species of plant (Geber 1990; Prati & Schmid 2000; Ronsheim & Bever 2000), though not in all studies (Weis, Hollenbach & Abrahamson 1987). In *Allium vineale*, Ronsheim & Bever (2000) found a strong negative genetic correlation between sexual flower traits and asexual bulbil traits. Geber (1990) found a negative genetic correlation between growth and fecundity in *Polygonum arenastrum*. The trade-off between sexual and clonal reproduction has been particularly well studied in *Ranunculus reptans* where it has been established

that there is heritable variation in both flowering and clonal traits (van Kleunen, Fischer & Schmid 2002; Fischer, van Kleunen & Schmid 2004). In this species, there is a negative genetic correlation between allocation to flowering traits and clonal traits, which is modified by competition (Prati & Schmid 2000; van Kleunen, Fischer & Schmid 2002; Fischer, van Kleunen & Schmid 2004).

The genetic correlations that we have documented suggest that there are genetically determined life-history strategy trade-offs in the BCCIL population of *F. ovina*. They indicate an axis of phenotypes that range from dense small tussocks with few seeds, a more perennial phenotype, to few-tillered large-leaved tussocks with many seeds, a more annual phenotype. This continuum fits with the stress-tolerator–ruderal axis of life-history strategy framework proposed by Grime *et al.* (1997) and developed in Grime (2001). Specifically, perennial species possess a tendency for asexual vegetative reproduction and a stress-tolerant strategy, and annual plants invest heavily in sexual reproduction allowing a ruderal strategy that facilitates exploitation of disturbed habitats (Grime 2001). This finding also corresponds with the phenotypic trade-off that we observe in an inability of plants to produce both a high number of tillers and a high number of seeds (Figure 3.5 **C**).

The extent to which genetic correlations may potentially constrain evolutionary responses depends in part on the direction and strength of selection in relation to the genetic correlation (Conner 2012). Providing that the correlation is less than one, evolution will not be prevented completely, although its progress will be substantially slowed, even when selection is antagonistic to the direction of a genetic correlation (Blows & Hoffman 2005). However, genetic trade-offs and the effects of pleiotropic genes can themselves evolve, which makes predicting

evolutionary responses very complex (Roff & Fairbairn 2012; Pavličev & Cheverud 2015).

Environmental heterogeneity is an important factor contributing to the maintenance of genetic variation within populations and is also an important factor determining how selection acts on genetic architecture within a population (Sgrò & Hoffmann 2004; Byers 2005). The habitat at BCCIL is highly spatially heterogeneous, with fine-scale variation in soil depth, pH and water and nitrogen availability (Fridley *et al.* 2011). In our study we have only measured heritability and genetic architecture under one specific set of environmental conditions, and these may well be altered in different environments, modifying potential evolutionary responses (Etterson 2004; Byers 2005). The next step, in this particular population, would be to set up experimental crosses among plants from drought and control treatments separately, and then to grow these plants under drought-treated and control (moist) environments. Multivariate comparison methods could then be used to compare the **G**-matrix between the populations, under these different environments. Such a study would allow us to understand the extent to which genetic correlations in the BCCIL population of *F. ovina* are environmentally labile, and enable distinct environmentally-defined evolutionary trajectories (Walsh & Blows 2009; Aguirre *et al.* 2014).

Maternal effects on the phenotype represent a further important factor for understanding the potential for evolutionary responses to climate change in our system. Early studies treated maternal effects as a source of variation simply to be accounted for in models (Wade 1998). However, it is now recognised that maternal effects are themselves subject to selection and can act as an important mechanism

for adaptive responses (Mousseau & Fox 1998; Shaw & Byers 1998; Galloway & Etterson 2007; Galloway, Etterson & McGlothlin 2009). Maternal genetic effects generate a time lag between the source of genetic variation, in the mother, and the expression of phenotypic variation, in the offspring (Galloway, Etterson & McGlothlin 2009). This can change evolutionary responses in unexpected ways, impeding or reinforcing the direction of selection (Wade 1998; Galloway, Etterson & McGlothlin 2009; Wolf & Wade 2016). It was far beyond the scope of this study to disentangle the influence of maternal genetic, and maternal environmental, effects on phenotypes. However, it seems likely, given the size of the maternal effects that we have reported, that they could be an important source of variation for adaptive evolutionary responses in this population.

3.6.3 Evolutionary responses to climatic selection at BCCIL

Despite the presence of significant heritable genetic variation, we found no evidence for evolutionary differentiation of plant phenotypes between climate treatments at BCCIL in any of the traits studied. This is contrary to evidence from other studies on this population of *F. ovina*, which have found that climatic selection has caused phenotypic and genetic differentiation between plants from the drought and control plots (Ravenscroft, Whitlock & Fridley 2015 and R. Whitlock, personal communication). In the *parent microcosm experiment,* an experiment in which clonal replicates of plants from the parent clonal library were grown in a common environment for 3 years, plants from drought plots had a smaller leaf area and fewer flowering tillers than those from the control plots (R.

Whitlock, personal communication). We do not see these same differences in this study. One possible reason for this disparity is that the two experiments were conducted in very different growing conditions. In the heritability experiment *F. ovina* were grown in very small pots, 5 cm × 5 cm × 5 cm, however in the parent microcosm experiment the plants were grown in large 2 litre pots. The small pots that we used in the heritability experiment represent a potentially more nutrient-limited environment, which may be representative of the conditions found in the shallowest soils at BCCIL. The nutrient limitation has resulted in the plants' being much smaller than those grown in the parent microcosm experiment, producing many fewer flowers and tillers. Evolutionary responses may be expressed differently in different edaphic environments. Another key difference between the two experiments is that in the heritability experiment we grew plants in the absence of competition, whereas in the parent microcosm experiment *F. ovina* was grown within a microcosm community of other species. Studies are increasingly showing that biotic interactions can be important for the detection and magnitude of adaptation to climatic conditions (Bischoff *et al.* 2006; Tomiolo, van der Putten & Tielbörger 2015).

3.6.4 Caveats and weakness of this study

A key weakness of our study was in the incorporation of measures of flower and seed number. The flowering data from this population suggests there is a range of different life-history strategies: some plants produce flowers every year, others produce flowers in some years, and others very rarely flower (over the course of

this experiment around one quarter of plants never flowered). The sporadic nature of flowering in *F. ovina* made it difficult to select a suitably representative way to incorporate these measures into the analyses without having to reduce significantly the size of the dataset. For this reason we measured flowering and seed number as a total across three years' growth. However, this clearly misses much of the subtlety in the way individual plants chose to invest in flowering in any given year, and the importance of these reproductive phenotypes for plant total fitness**.**

Estimates of heritability and genetic architecture can be biased by assortative mating, as standard quantitative genetics approaches assume random mating (Lynch & Walsh 1998). We know from Chapter 2 that mating in this population is assortative by flowering time, and our estimates of heritability may be biased because of this. However, Fox (2003) argued that quantitative genetic measures derived from studies that have manipulated mating among parents to enforce random mating do not reflect gene flow within natural populations where mating does occur assortatively. Our approach, allowing mating through natural wind pollination, may therefore provide estimates of heritability and genetic architecture that more realistically reveal the levels actually occurring in the natural population.

3.6.5 Conclusions

We have demonstrated narrow-sense heritability in morphological and reproductive traits in the BCCIL population of *F. ovina* and shown that there is a large maternal component to phenotypic variation within this population. We have

also quantified the genetic architecture among traits and identified a negative genetic correlation between asexual (tiller number) and reproductive (number of seed) traits that suggests a genetically determined trade-off in life-history strategy. These results suggest that sexual, asexual and competitive components of fitness will not be able to respond simultaneously through evolutionary responses to climate change. We found no evidence of phenotypic differentiation between plants of drought and control ancestry at BCCIL, when grown in a common environment, suggesting that evolutionary responses to climatic selection are not occurring in this population. The presence of heritable genetic variation within this population represents a basis for adaptive evolutionary responses to climate change in *F. ovina*; however, negative genetic correlations could potentially constrain these responses, limiting the suite of possible adaptive strategies that could evolve.

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4 Chapter 4. Intraspecific variation in genome size in *Festuca ovina* and its adaptive significance

4.1 Abstract

Genome size may vary among individuals of the same species, but its determinants are complex. The extent to which variation in genome size is a direct product of selection, as opposed to neutral factors, remains unclear. We add to the small number of studies on this topic by studying intraspecific variation in genome size in *Festuca ovina* collected from the Buxton Climate Change Impacts Laboratory (BCCIL), where natural grassland has been subjected to experimental climate change for 17 years. Genome size was measured using flow cytometry in plants collected from drought-treated and control plots at BCCIL. We examined whether long-term experimental climate change has altered genome size and whether intraspecific variation in genome size is associated with any particular adaptive phenotypes in *F. ovina*. Within this population, genome size varies by a maximum of 1.278-fold. We have shown that the range of genome size in offspring is larger than that in the natural population, which suggests selection in the natural environment is removing large and small sized genomes. There was no evidence that long-term climatic selection has altered genome size between the drought and control plots at BCCIL, but genome size is correlated with soil depth at BCCIL. Genome size is not correlated with cell size, flowering time or biomass, suggesting that intraspecific variation in genome size at this scale is non-adaptive.

4.2 Introduction

When studying adaptive responses to climate change, we typically consider how selection acts on standing genetic variation that is associated with particular adaptive phenotypes (Shaw & Etterson 2012; Franks, Weber & Aitken 2014). However, genome size is another component of the genetic variation of an organism and is associated with different phenotypes and life-history strategies (Grime & Mowforth 1982; Knight, Molinari & Petrov 2005; Greilhuber & Leitch 2013), as well as influencing the composition of plant communities (Šmarda *et al.* 2013; Guignard *et al.* 2016). Intraspecific variation in genome size has been less studied, but has also been shown, in a few cases, to be associated with environmental variables such as aridity (Kalendar *et al.* 2000; Bureš *et al.* 2004). As such, variation in genome size is potentially an important component influencing the evolutionary responses of plants to climate change.

Genome size refers to the total amount of DNA in a cell nucleus (Greilhuber 2005; Leitch & Leitch 2013). At the species level, genome size correlates with numerous plant traits including cell cycle duration and cell size (Van't Hof & Sparrow 1963; Bennett, Lewis & Harberd 1977), stomatal density (Beaulieu *et al.* 2008; Knight & Beaulieu 2008), seed mass (Knight & Ackerly 2002; Beaulieu *et al.* 2007), specific leaf area (Knight, Molinari & Petrov 2005; Kang *et al.* 2014), phenology (Grime & Mowforth 1982; Grime, Shacklock & Brand 1985) and breeding system (Albach & Greilhuber 2004). Studies have also found associations between genome size and a range of environmental and geographic variables including climate (Macgillivray & Grime 1995; Knight & Ackerly 2002; Knight, Molinari &

Petrov 2005), altitude and latitude (reviewed by Knight, Molinari & Petrov 2005), and pollution levels (Vidic *et al.* 2009; Temsch *et al.* 2010). The relationships between genome size and environmental variables are complex, with genome size often appearing to constrain correlations with environmental gradients, as opposed to linearly correlating with them (Knight, Molinari & Petrov 2005).

Of particular interest, in relation to the responses of plant communities to climate change, are studies that have shown how genome size influences life-history strategy or plant community composition in relation to the environment. For example, Grime & Mowforth (1982) showed that spring growth was negatively correlated with genome size in the British flora. They suggested that species with large genomes carried out their cell division in the previous summer, when the process was not temperature-limited, and then grew more quickly in early spring when water became available and they could grow by rapid expansion of their large cells. This was supported by a later study, which found that the rate of leaf expansion was associated with genome size (Grime, Shacklock & Brand 1985). More recently, the studies of Šmarda *et al.* (2013) and Guignard *et al.* (2016) have used long-term plots at the Rengen Grassland Experiment, Germany, and the Park Grassland Experiment, Rothamsted, UK, to demonstrate that genome size influences life-history strategy and alters plant community composition and ecosystem function. These two studies showed that an increased supply of nutrients had shifted community composition within those plots, and that this shift was associated with large genome-sized plants with competitive life-history strategies. The high phosphorus and nitrogen requirements that come from having large genomes are an important component governing plant life-history strategy

and, as a result, community composition. Together, these three studies demonstrate the way genome size can influence a species' life-history strategy under particular environmental conditions.

Genome size also varies within species (Greilhuber 2005; Šmarda & Bureš 2010). However, methodological problems with early genome size estimation techniques have led to many reported cases of intraspecific variation in genome size being subsequently rejected as inaccurate (Greilhuber 2005; Šmarda & Bureš 2010). In recent years, the increased use of flow cytometry, the implementation of a consistent internal standard and the demonstration of double peaks from co-chopped samples has provided convincing verification of intraspecific variation in genome size (Šmarda & Bureš 2010; Greilhuber & Leitch 2013), in species including *Koleria macrantha* and *K. tristis* (Pecinka *et al.* 2006), *Bituminaria bituminosa* (Walker, Moñino & Correal 2006), *Hieracium brachiatum* (Suda *et al.* 2007) and *Anthoxanthum* species (Chumová *et al.* 2015).

A few studies have documented relationships between intraspecific variation in genome size and environmental or geographic variables. For example, in *Allium oleraceum,* genome size was shown to vary by latitude and longitude (Duchoslav, Šafářová & Jandová 2013). Similarly in *Zea mays*, Díez *et al.* (2013) showed that variation in genome size was correlated with geography and climate variables, although the patterns were inconsistent, differing between maize cultivars and wild populations. A particularly well-characterised study system, with respect to genome size and climate, is "Evolution Canyon", Mount Carmel, Israel (Nevo 2012). Here, intraspecific variation in genome size has been identified in four species, *Hordeum spontaneum* (Kalendar *et al.* 2000), *Ceratonia siliqua* (Bureš *et al.*

2004), *Lotus peregrinus* (Gasmanová *et al.* 2007), and *Cyclamen persicum* (Pavlíček *et al.* 2008). The two slopes of Evolution Canyon have very different microclimates, which has enabled researchers to study how genome size varies between the south-facing, drier slope in comparison to the north-facing, wetter slope (Nevo 2012). In *C. siliqua* genome size was significantly larger in trees on the drier south-facing slope compared to the wetter north-facing slope, and genome size was significantly negatively correlated with leaf length and tree circumference (Bureš *et al.* 2004). It was also found that average genome size in *H. spontaneum* was larger on the drier south-facing slope (Kalendar *et al.* 2000). Furthermore, it was shown that the highest copy number of the retrotransposon *BARE*-1, which is positively correlated with genome size, was found at the most arid sites (Vicient *et al.* 1999; Kalendar *et al.* 2000). No significant differences were found between the genome sizes from the two slopes of two other species, *Lotus peregrinus* (Gasmanová *et al.* 2007) and *Cyclamen persicum* (Pavlíček *et al.* 2008). These studies demonstrate that intraspecific variation in genome size can be ecologically adaptive, but whether this is typical to most species is unknown.

The fine-leaved fescues of the genus *Festuca* are one of the best-studied cases of intraspecific genome size variation. Intraspecific variation in genome size has been demonstrated in four species (*F. pallens, F. polesica, F. rupicola, F. vaginata*) with especially detailed research carried out on *F. pallens* (Šmarda 2006; Šmarda & Bureš 2006; Šmarda, Bureš & Horová 2007; Šmarda *et al.* 2008; Šmarda *et al.* 2010). In *F. pallens*, intraspecific variation in genome size has been documented in both diploid and tetraploid populations, with maximum differences of 1.170-fold and 1.164-fold, respectively (Šmarda & Bureš 2006; Šmarda, Bureš &

Horová 2007). Genome size in *F. pallens* varies with latitude and longitude across its geographic distribution (Šmarda & Bureš 2006). However, no correlations between intraspecific variation in genome size and microclimate conditions were found in a fine-scale study of a single tetraploid population (Šmarda, Bureš & Horová 2007). Genome size has also been demonstrated to be under stabilising selection in *F. pallens*. Šmarda *et al.* (2010) grew seeds of *F. pallens* under no competition versus high competition and monitored their survival and growth. They found a 0.82-fold reduction in genome size variation in surviving plants under competition. However, the mechanism underlying the observed selection is uncertain; it is not clear whether genome size itself, or some other correlated trait, is the target of selection (Beaulieu 2010; Šmarda *et al.* 2010).

In this chapter, we examine intraspecific variation in genome size in the fine-leaved fescue *F. ovina*, using individuals collected from a long-term climate manipulation experiment at the Buxton Climate Change Impacts Laboratory (BCCIL), which allows us to examine whether genome size has altered under climatic selection. We collected *F. ovina* plants from drought and control plots at BCCIL, and used flow cytometry to measure ploidy and genome size. We investigated whether genome size has altered between the treatments at BCCIL following 17 years of climatic selection and assessed whether genome size is correlated with soil depth, an important source of habitat heterogeneity at BCCIL. Finally, we examined whether genome size correlates with any phenotypic traits that are potentially adaptive under climate change. We found a maximum 1.278-fold variation in genome size within the population of *F. ovina* at BCCIL, but no evidence that

genome size has altered between drought and control treatments under long-term climatic selection.

4.3 Methods

4.3.1 Study site and species

This work uses *F. ovina* collected from the BCCIL study system (specifically drought-treated and control plots). At BCCIL, 3 m × 3 m plots of calcareous species-rich grassland have been subjected to climate manipulation treatments annually since 1994 (see Chapter 1, Section 1.4 for further details on BCCIL). In brief, a range of climate treatments are applied at BCCIL including summer drought, winter warming and supplementary summer rainfall, along with factorial combinations of these and non-manipulated control plots (Grime *et al.* 2000). Plots are replicated five times in a randomised block design including control plots. Our work focuses on the drought treatment (in comparison to the control treatment), which has seen the greatest quantity of species-level changes in comparison with the other treatments being applied at BCCIL (Fridley *et al.* 2011). The drought treatment is applied annually, through July and August, by using automatic rain shelters, which cover the plot when it is raining, and return to an off-plot position when the rain stops. This results in a significant reduction in the water potential in the surface soil at the end of the drought treatment (-1100 kPa in drought plots vs. -20 kPa in control plots, at 5 cm depth; see Figure 4 in Fridley *et al.* 2011).

4.3.2 Collection and propagation of F. ovina clonal lines from BCCIL

In July 2010 individuals of *F. ovina* were collected from the plots at BCCIL by R. Whitlock, after 17 years of climate manipulation (Full details found in Chapter 2, Section 2.3.2). Thirty individuals were collected from the drought and control treatments (six individuals per plot, per treatment). Small bunches of 4–8 connected tillers were recovered from each sampled plant in the field. Soil depth was recorded at each sampling site. Plants were established in 3 L pots containing a 3:1 mix of John Innes No. 1 potting compost and medium grade Perlite (LBS Horticulture). Plants were housed in purpose-built raised "bays" at Ness Botanic Gardens, University of Liverpool, UK. They were maintained by seed head removal during the summer, biomass clipping in September and supplementary watering as required. This set of 59 clonal lines (one individual died following collection) is referred to hereafter as the *parent clonal library* [4].

During the flowering period of 2012 (May-June) we created an F1 progeny array (full methods can be found in Chapter 2, Section 2.3.3). The individuals in the parent clonal library were allowed to mate through natural wind pollination. Seeds were collected from the 58 parent plants that produced seed, and from these bulk collections of seed, 16 seeds from each parent clone were selected at random and germinated. Of the seeds that germinated, eight seedlings from each parental clone were planted on and established. These individuals are maintained as clonal lines at Ness Botanic Garden following the same regime as the parent clonal library, hereafter called the *offspring clonal library*.

[4] Terms in italics are defined in the glossary

4.3.3 Plant materials

Leaf tissue, in the form of two tillers per individual, was collected for measurements of ploidy and genome size. Leaves were stored in sealed plastic bags containing wet tissue paper in a fridge until processing. Leaf tissue for parent plants was collected from the *F. ovina parent clonal library,* leaf tissue for offspring plants was collected from the heritability experiment which was derived from the offspring clonal library (methods described in Chapter 3, Section 3.3).

4.3.4 Flow cytometry

In April 2014 the ploidy of the parent and progeny clonal lines were measured using flow cytometry. Measurements were taken at the Jodrell Laboratory, Royal Botanic Gardens, Kew. For each sample, a basal section of young leaf of *F. ovina* was chopped along with a standard of *Petunia hybridum* (2C = 2.85 pg) using a razor blade in a petri dish containing 1000 µl 'General Purpose Buffer' (GBP) (Loureiro *et al.* 2007) supplemented with 3% PVP-40. The mixture was filtered through a 30 µm nylon mesh (Partec), stained with 50 µl propidium iodide (Sigma, 1 mg mL^{-1}) and left on ice for 15 minutes. Three thousand cells were analysed in each measurement. Relative DNA content was then calculated for each sample compared to the internal standard. DNA content for each sample was calculated as follows:

$$\text{DNA content} = \frac{\text{Mean peak of the sample}}{\text{Mean peak of the standard}} \times \text{2C DNA content of the standard}$$

The measurements, originally intended to determine ploidy, indicated substantial intraspecific variation in genome size, as well as identifying individuals with extreme genome sizes. Therefore, a subsequent analysis of genome size was conducted on all the parent plants, offspring representing the extremes of the range of variation, and an offspring individual identified as an outlier.

In May 2015 the relative DNA content of the parent plant clonal lines was determined using propidium iodide stained flow cytometry, following the protocol described above, with the following amendments. For each sample a basal section of young leaf of *F. ovina* was chopped along with a standard of *Petroselinum crispum* (2C = 4.5 pg). The relative nuclear DNA content was estimated by recording at least 1,000 cells per peak on a Partec CyFlow Space flow cytometer (Sysmex, UK). Measurements took place over the course of three weeks, and samples were measured in a randomised order. Three leaves were measured per clonal line and a mean DNA content calculated as the average of these three values.

4.3.5 Chromosome counts

Chromosome counts were carried out by Dr Hugh McAllister. The parents and offspring for individuals with the largest and smallest genome size in the main range of variation (as measured using flow cytometry), along with individuals with outlier genome size measurements, were selected for chromosome counting. Chromosome counts were measured on fresh rapidly-growing root tips. Full methods for chromosome counting can be found in Appendix 4, Section A4.1.

4.3.6 Phenotypic measurements

4.3.6.1 Guard cell size

The length of a guard cell was used as a measure of cell size. Guard cell length does not change regardless of whether the stoma is open or closed (Willmer & Fricker 1996). The longest leaf on each plant from the parent clonal library was collected and sealed in a plastic bag with wet tissue paper to maintain leaf moisture content. Leaves were stored in a fridge prior to processing. The leaf was cut in half along its length under a dissection microscope, allowing the inside edge of the leaf, where the stomata are located, to be visible. The inside edge of the leaf was painted with clear nail varnish and left to dry for five minutes. A piece of Sellotape was placed on top of the nail-varnished area and quickly peeled off. This transferred the nail varnish imprint onto the Sellotape, which was then stuck to a microscope slide and viewed at 40 × magnification on a UNILUX-12 light microscope (Kyowa Optical Co. Ltd). A picture was taken using a Canon ESO 1000D digital SLR camera mounted to the microscope. Images of the guard cells (Figure 4.1) were analysed using ImageJ (Abramoff, Magalhaes & Ram 2004). Measurements were calibrated against an image of a stage micrometer. The lengths of, on average, 15 guard cells were measured for each leaf, and an average guard cell length calculated for each plant.

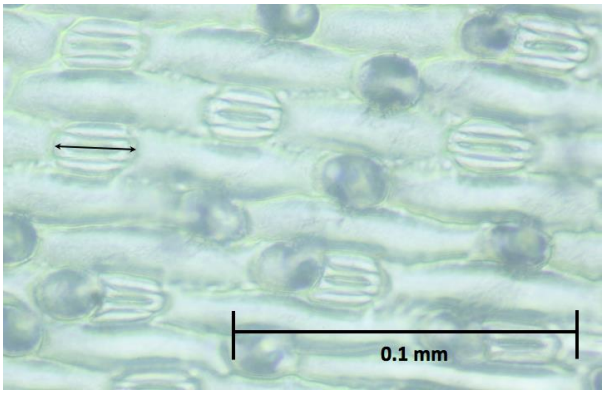

Figure 4.1 Image of guard cells taken at 40 × magnification. Arrow indicates the length of a guard cell measurement.

4.3.6.2 Phenology

Flowering time data were taken from another experiment in which clonal replicates of the plants from the parent clonal library were grown in a common environment at Ness Botanic Gardens, the *parent microcosm experiment*. Flowering time data were collected during the summer of 2013. Flowering time was measured as the date of first anthesis (at which the first anther is visible in any spikelet). Full methods for the collection of flowering time data are provided in Appendix 2, Section A2.7.

4.3.6.3 Biomass

Biomass data also came from the *parent microcosm experiment*. Vegetative biomass above 25 mm was collected in September 2013, dried for 1 week at 55°C and weighed. The vegetative biomass was the mean of four or five replicates for each clonal line.

4.4 Statistical analysis

4.4.1.1 Generalised linear mixed-effects models specifications

To examine the relationship of intraspecific variation in genome size with climatic and phenotypic variables we used generalised linear mixed-effects models (GLMMs) carried out in R (R Development Core Team 2008) in MCMCGLMM (Hadfield 2010). MCMCGLMM uses a Bayesian approach to implement models; a discussion of the benefits of a Bayesian approach can be found in Chapter 2, Section 2.5.2. Unless specified otherwise, GLMMs had the following specifications. Models were run for 1,300,000 iterations, with a burn in of 300,000 and a thinning interval of 1,000, resulting in a sample size of 1,000. The prior for variance components was a non-informative uniform improper prior distribution on the standard deviation of the random effects (specified as $V = 1.0 \times 10^{-16}$, $nu = -1$ in MCMCGLMM), as recommended by Gelman (2006). For each model the sensitivity to starting parameters was tested by running the model three times with over-dispersed chain starting values using the parameter *start=list(QUASI=FALSE)*, and using the Gelman-Rubin diagnostic to assess convergence (Gelman & Rubin 1992). The blocking factor for plots at BCCIL was fitted as a fixed effect (5 levels) in models, and contrasts for the plot block variable were centred to allow estimation of the remaining parameters to a notional "average" block. We used the *autocorr* function to check the autocorrelation, which is the level of non-independence between successive samples of the chain. The autocorrelation between the first and second successive stored samples were checked and values below 0.15 were required for the model to be accepted following the recommendation of Hadfield (2012). Post-hoc

comparisons for particular parameter contrasts were made by re-levelling variable levels as needed. We report "*95% credible intervals"* which is the range within which we expect, with a probability of 0.95, the true parameter value to be located. Credible intervals were calculated using the *HPDinterval* function in MCMCGLMM.

4.4.1.2 i) Is there intraspecific variation in genome size in *F. ovina*?

Genome size measurements were obtained for 57 of the parent plants. Each genome size measurement is an average of three measurements of genome size for each plant. An analysis of the repeatability of the three genome size measurements taken for each plant is provided in Appendix 4, Section A4.2. One plant, '2936', had a much larger genome size than the others plants, and therefore this individual was excluded from all further analyses.

4.4.1.3 ii) Does genome size vary with climate treatment or soil depth at BCCIL?

To test whether intraspecific variation in genome size differed with respect to climate treatment and local soil differences at BCCIL we analysed genome size in a GLMM, fit in MCMCGLMM. Genome size was fitted with a Gaussian family. Climate treatment and mean soil depth at BCCIL were fitted as fixed effects. To examine whether the variation in genome size differed between deep and shallow soil we performed Levene's test. The cut-off between shallow and deep soil was set at the median soil depth for the dataset.

4.4.1.4 iii) Does genome size correlate with key phenotypic traits?

To test whether genome size is correlated with guard cell size at the intraspecific level we analysed guard cell size in a GLMM, fit in MCMCGLMM. Guard cell size was fitted with a Gaussian family. Climate treatment at BCCIL and genome size were fitted as fixed effects. No guard cell measurements could be obtained for one individual, so the dataset was reduced to N = 55. To test whether genome size was correlated with each of these phenotypic traits in *F. ovina*, we analysed flowering time and biomass data in independent GLMMs, fit in MCMCGLMM. Flowering time was fitted with a Gaussian family and biomass data were square root transformed and fitted with a Gaussian family.

4.5 Results

4.5.1 i) Is there intraspecific variation in genome size in F. ovina?

In total, ploidy was determined for 450 offspring and 57 parent plants, of which 449 offspring and 57 parents were identified as tetraploids (4*x*). One offspring individual, '2268-B3', was identified as having a very large 2C genome size, 14.70 pg, consistent with it being a hexaploid (6*x*). This was supported by its chromosome count 2*n* = 42 (Table 4.1). One parent individual, '2936', also had a considerably larger 2C genome size than the rest of the plants measuring 12.28 pg. However, chromosome counts supported this individual being a tetraploid and did not identify any extra chromosomes. Neither of the individuals with larger genome sizes was noticeably morphologically different to the tetraploid plants.

Table 4.1 Individuals of *F. ovina* on which chromosome counts were carried out, details of the count, and the reason that individual was selected.

Individual	Count	Description
2958	28 (26)	Parent plant with the smallest genome size measured
2940	26	Parent plant with the largest genome size measured, except for the outlier
2936	28	Outlier parent plant
2232-C3	28	Offspring plant with the smallest genome size measured.
2280-B3	28	Offspring plant with the largest genome size measured, except for the putative hexaploid.
2268-B3	42	The offspring plant with the largest genome size measured.

Chromosome counts carried out by Dr Hugh McAllister

The average 2C genome size of the parent plants was 9.85 pg; 9.81 pg with the outlier individual '2936' excluded. A maximum 1.278 fold difference in genome size was observed in the parent plants; this was reduced to a maximum difference of 1.061 fold with outlier individual '2936' excluded (Figures 4.2 & 4.3 **A**). Excluding the hexaploid individual, a maximum 1.202-fold difference in genome size was observed in the offspring plants (Figures 4.2 & 4.3 **B**). The offspring plants had a larger range of intraspecific variation in genome size than the parent plants, with the hexaploid and outlier parent individual excluded (Figure 4.2). The average coefficient of variation (CV) of the peaks for the standard was 2.523 while the average CV of peaks for the sample was 2.177.

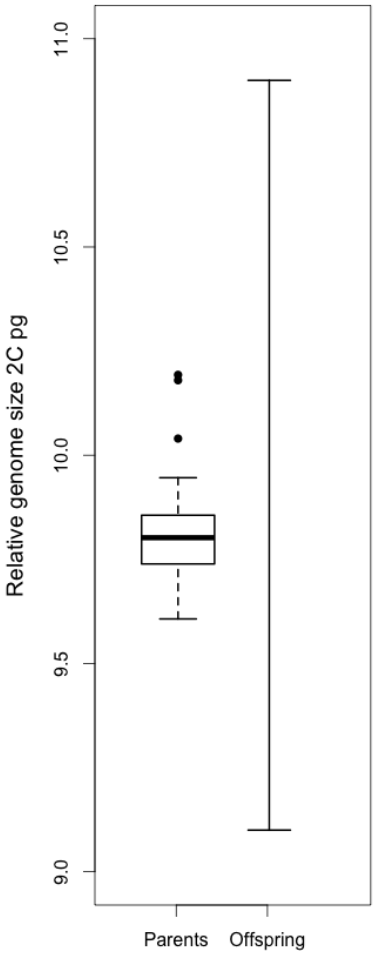

Figure 4.2 Relative genome size variation in parents and their offspring, excluding parent outlier '2936' and offspring hexaploid '2268-B3'.

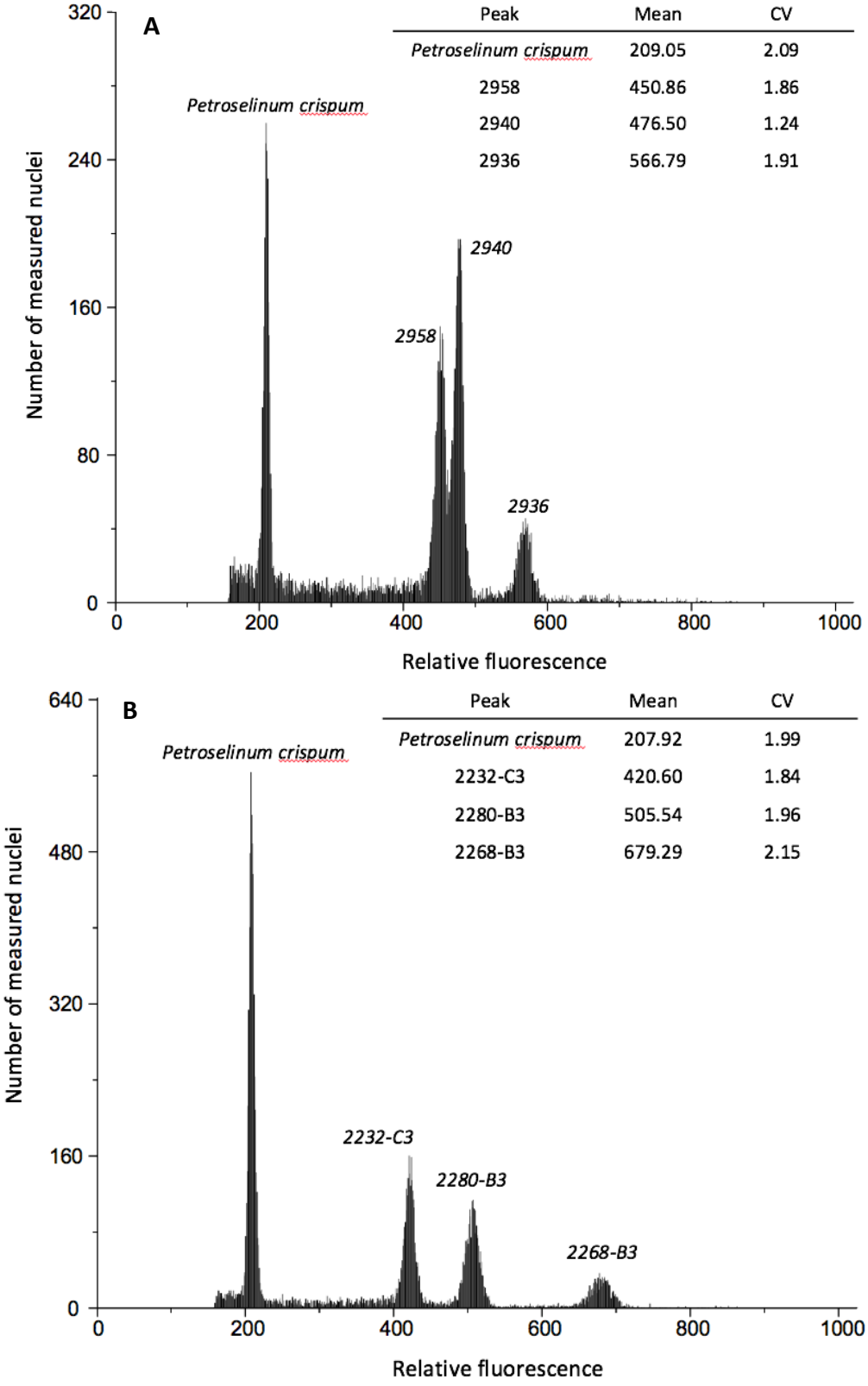

Figure 4.3 Differences in relative DNA content in simultaneously measured samples of *F. ovina* and a standard of *Petroselinum crispum* **A)** Maximal differences in plants from the parent clonal library **B)** Maximal differences in plants from the offspring clonal library. CV = coefficient of variation of the peak.

4.5.2 *ii) Does genome size vary with climate treatment or soil depth at BCCIL?*

There was no significant difference in the genome size of plants dependent on the treatment at BCCIL (*pMCMC* = 0.956), but there was a significant effect of soil depth (*pMCMC* = 0.022; Figure 4.4). Genome size was negatively correlated with soil depth; individuals with large genomes were more likely to be found in shallow soils than individuals with small genomes. Two individuals with large genomes appeared to be driving this pattern. Re-analysis with these two individuals excluded from the analysis found genome size was still negatively correlated with soil depth, although the association was less strongly supported (*pMCMC* = 0.066). There was no significant difference in the variance in genome size between shallow and deep soil (Levene's test; F = 2.441, *p* = 0.124).

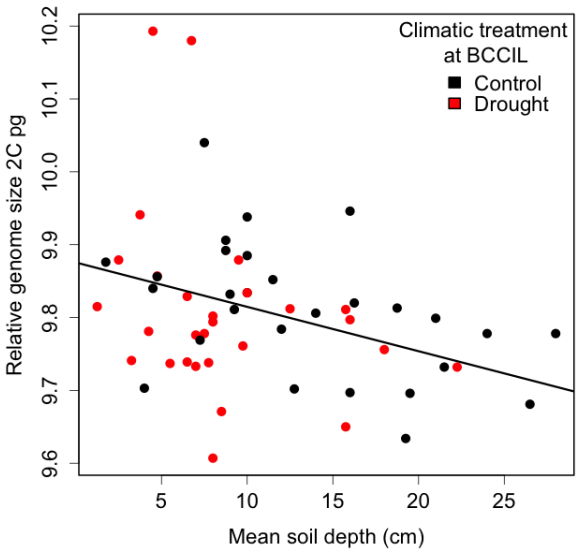

Figure 4.4 Relative genome size in *F. ovina* individuals collected from BCCIL in relation to the mean soil depth from which the plant was collected.

4.5.3 *iii) Does genome size correlate with key phenotypic traits?*

The average guard cell length was 25.8 µm. There was no significant difference in the average guard cell length dependent on treatment at BCCIL

(*pMCMC* = 0.146; Figure 4.5 **A**). There was no relationship between guard cell size and genome size (*pMCMC* = 0.560; Figure 4.5 **B**). There was no relationship between genome size and minimum flowering time (*pMCMC* = 0.186) or between genome size and vegetative biomass (*pMCMC* = 0.280).

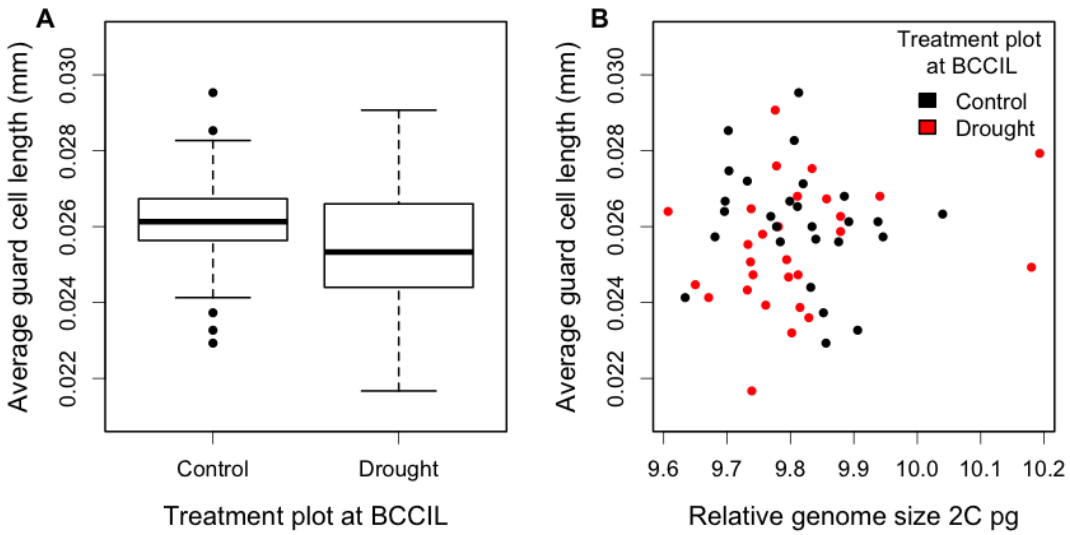

Figure 4.5 Guard cells measurements on parent plants. **A)** Boxplot of the average guard cell lengths based on the treatment plot at BCCIL that the plant was collected from. In the box plot the box represents the first and third quartile, whiskers represent ± 1.5 × interquartile range, with points lying outside the range of the whiskers being outliers. **B)** Average guard cell length in relation to the relative genome size of the plant.

4.6 Discussion

In this chapter we measured genome size in *F. ovina* collected from BCCIL. We then used this data to determine whether intraspecific variation in genome size has altered between treatments at BCCIL and whether it is correlated with any

particular phenotypes. We have documented a maximum of 1.278 fold variation in genome size in the BCCIL population of *F. ovina* and observed that, with the exception of an outlier individual, the range of genome size is larger in offspring plants, than in the parent population. We found no evidence that genome size has altered between the treatments at BCCIL but we have identified a significant negative relationship between intraspecific variation in genome size and soil depth at the BCCIL sampling site. We also did not identify a correlation between genome size and any of the phenotypic traits measured here: guard cell size, flowering time or vegetative biomass. These results demonstrate genuine intraspecific variation in genome size in *F. ovina*, but this has not been selected for under long-term drought, suggesting that it is not ecologically adaptive.

4.6.1 i) Is there intraspecific variation in genome size in F. ovina?

We have documented a maximum 1.278 fold variation in genome size in *F. ovina* collected from BCCIL and a 1.202 fold variation in the genome size of the F1 progeny. The level of variation in genome size that we documented is comparable with the maximum differences documented in other fine-leaved fescues: *F. pallens* 4*x* = 1.164-fold, 2*x* = 1.170-fold, *F. polescia* 2*x* = 1.055-fold, *F. rupicola* 6*x* = 1.038-fold, and *F. vaginata* 2*x* = 1.042-fold (Šmarda 2006; Šmarda & Bureš 2006).

The use of flow cytometry to screen greater number of individuals has resulted in rare ploidies being increasingly identified within populations (Pellicer *et al.* 2012; Husband, Baldwin & Suda 2013). In this study we identified one offspring individual '2268-B3' as a hexaploid (6*x*). All of the parent plants were identified as

tetraploids, and so this individual appears to illustrate the spontaneous generation of a hexaploid. Other study systems have recorded the generation of hexaploid offspring from tetraploid parents in *Beta vulgaris* (Hornsey 1973) and *Achillea borealis* (Ramsey 2007), and it is mostly likely the result of irregular gametogenesis resulting from the combination of a reduced 2*x* gamete with an unreduced 4*x* gamete (Ramsey & Schemske 1998; Ramsey 2007).

Although it was outside the scope of this study to take precise measurements of genome size for each of our offspring plants, by using a high-throughput method to measure ploidy level we were able to identify the range of genome size variation present between them. This allowed us to identify individuals at the ends of the range, and identify outlier individuals. We have documented that, with the exclusion of one parent outlier individual, there was a greater range of genome sizes in offspring than in parent plants; parents = 1.061 fold, offspring = 1.202 fold. This corresponds closely with the work of Šmarda *et al.* (2008) who also found that offspring plants have a greater range of genome sizes than parents, and also demonstrated that the considerable variation (1.119-fold) could be generated in a single generation. In a later study, Šmarda *et al.* (2010) demonstrated that this pattern was most likely the result of stabilising selection removing the smallest and largest genomes. Our results suggest that a similar process is occurring within the population at BCCIL. In each new generation a range of variation in genome size is generated in the offspring, but selection then acts on these to allow only a small proportion to survive, although occasionally extreme-sized genomes may survive, such as our sample 2936, the outlier individual in terms of genome size. This

suggests that selection is acting at some level on genome size within the BCCIL population.

Variation in genome size in plants is primarily the result of repetitive DNA, particularly transposable elements, which can account for more than half of the genome (SanMiguel *et al.* 1998; Vicient *et al.* 1999; Gaut 2002; Bennetzen, Ma & Devos 2005; Meagher & Vassiliadis 2005; Hawkins *et al.* 2006). Retrotransposons are known to be a particularly important cause of genome size variation in grasses (Gaut 2002). Another contributor to variation in genome size may be gene content or copy number, although as a much smaller factor (Gaut 2002).

4.6.2 ii) Does genome size vary with climate treatment or soil depth at BCCIL?

There is no evidence that long-term climatic selection at BCCIL has selected for different genome sizes under the drought or control treatments. However, we have found that intraspecific variation in genome size varies with soil depth at the BCCIL. Several studies have investigated changes in genome size following long-term experimental plot manipulation treatments. In a study highly comparable to this chapter, Pellicer *et al.* (2010) investigated differences in genome size in six Mediterranean plant species at a long-term climate change experiment conducted in the Garraf Massif, Catalonia. They found no significant differences in genome size between warming, drought or control treatments, following seven years of the applied treatments. Our study more than doubles the length of time under climatic selection, with plants collected following 17 years under a drought treatment. We also did not find significant differences in genome size between drought and

control treatments, which, given the range of variation we observe in the offspring data, is not for lack of variation in genome size.

We found that genome size is negatively correlated with soil depth. Individuals with larger genomes were more likely to be found in shallow soils, and individuals with small genomes were more likely to be found in deep soils, although it should be noted that this pattern is strongly influenced by two individuals with large genomes. Many microclimatic and biotic variables change with soil depth including water availability, nutrient availability, soil temperature and competitive intensity (Fridley *et al.* 2011; Fridley *et al.* 2016). This makes it difficult to speculate whether a particular variable is responsible for the pattern that we see here. Many studies of intraspecific variation in genome size have found associations between species microsite or geographic location (latitude, longitude or altitude), including in *H. spontaneum* (Kalendar *et al.* 2000), *C. siliqua* (Bureš *et al.* 2004), *F. pallens* (Šmarda & Bureš 2006), *Allium oleraceum* (Duchoslav, Šafářová & Jandová 2013) and *Z. mays* (Díez *et al.* 2013). However, several of these studies have struggled to identify associations between specific climatic variables related to location.

In *F. pallens*, genome size was shown to correlate with latitude and longitude, with greater genome size in the south-east, and smaller genome sizes in the north-west of its Central European range (Šmarda & Bureš 2006). There was also an association between larger sized genomes and relict habitats. However, in a later study on a single population Šmarda, Bureš & Horová (2007) found no association between genome size and microclimatic conditions. Similarly, in *A. oleraceum*, strong positive correlations were found between genome size and latitude and longitude, but only weak associations found between genome size and

specific climatic variables (Duchoslav, Šafářová & Jandová 2013). In *Z. mays*, genome size has been shown to be correlated with altitude, longitude, temperature and precipitation factors (Díez *et al.* 2013). However, which climatic or geographic factors were most important varied between landraces and teosintes (wild subspecies of *Z. mays*). Other studies, such as those on *Lotus peregrinus* and *Cyclamen persicum,* studied at 'Evolution Canyon', Israel, have found no evidence of associations between genome size and environmental variables (Gasmanová *et al.* 2007; Pavlíček *et al.* 2008).

4.6.3 iii) Does genome size correlate with key phenotypic traits?

We found no evidence that genome size was correlated with guard cell size, flowering time, or vegetative biomass. Although correlations between genome size and phenotype are well documented at the interspecific level, at the intraspecific level correlations between phenotype and genome size have been less consistent (Greilhuber & Leitch 2013). For example, in *F. pallens* genome size was found to correlate with the rate of seedling development (Šmarda *et al.* 2008), although no relationship was found between genome size and seed mass or biomass (Šmarda & Bureš 2010). Genome size in *Corchorus olitorius* is negatively correlated with flowering date and positively correlated with seed surface area (Benor, Fuchs & Blattner 2011). In *C. siliqua* genome size is significantly negatively correlated with leaf length and tree circumference (Bureš *et al.* 2004). Conversely, in *C. persicum*, Pavlíček *et al.* (2008) found no relationship between genome size and any of the phenotypic traits measured.

The mechanisms underlying the association between genome size and phenotype are poorly understood. Currently the majority of studies on the relationship between genome size and phenotype are correlational. This makes it difficult to assign causality or to determine a specific mechanism resulting in the observed relationships (Greilhuber & Leitch 2013). Two main theories exist. The nucleotype theory centres on the relationship between DNA content and its effect on cell volume and the duration of cell cycle replication volume (Meagher & Vassiliadis 2005). The regulatory theory focuses on the influence of specific types of repetitive DNA which can regulate gene expression (Meagher & Vassiliadis 2005). For example, particular transposable elements may be adaptive under stressful conditions and may also increase genome size by their presence (Kalendar *et al.* 2000; Casacuberta & González 2013). At the species-level, genome size has been clearly shown to correlate with, or constrain, many aspects of plant phenotype (Knight & Beaulieu 2008; Greilhuber & Leitch 2013). However, at the intraspecific level, patterns are much more inconsistent.

In our study, the greater range of genome size that we found in the offspring plants (with our parent outlier excluded), compared to the parent plants collected from the field, suggests that there is some selection acting on genome size within the BCCIL population. Equally, the presence of the outlier individual suggests that this force is weak and that individuals with extreme genomes only establish in the wild infrequently (Beaulieu 2010). Given that we found no correlation between genome size and any of the phenotypic traits studied, it is unclear on which trait selection may be acting. In their study on *F. pallens*, Šmarda & Bureš (2010) showed that growth under strong intraspecific competition resulted

in stabilising selection on genome size, although this was a weak selective force. At the BCCIL site shallow soils are likely to be under less intense competition than deep soils. Therefore, one possible reason for the relationship we observe between soil depth and genome size is that there is less competition in shallow soils, which allows larger genomes to establish without being selected against and removed. Conversely, in an earlier study on *F. pallens* Šmarda *et al.* (2008) found a positive correlation between the rate of seedling development and genome size, which they believed may offer plants a competitive advantage. If a larger genome size conferred a competitive advantage through development rate then it might be expected that individuals with large genomes would be found in deep soils, under more intense competition. Further work on this population will be required to determine whether the relationship between genome size and soil depth is the result of the sampling of this dataset, an association between genome size and a phenotype not measured here, or accumulation of repetitive DNA advantageous in shallow soils.

4.6.4 Conclusions

We have documented intraspecific variation in genome size in the BCCIL population of *F. ovina*. We have shown that the range of genome size in offspring is larger than that in the natural population, which suggests selection is removing large and small sized genomes. We found no evidence that genome size has changed under long-term climatic selection at BCCIL, but have documented a negative relationship between genome size and soil depth. Genome size is not

correlated with cell size, flowering time or vegetative biomass. These results indicate that genome size is not an important factor for evolutionary responses to drought in *F. ovina*, but may influence fine-scale microsite location through some unknown mechanism.

4.7 References

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5 Chapter 5. Adaptive evolutionary responses to climate change in *Festuca ovina* and their ecological consequences

5.1 Abstract

Recent studies have documented evolutionary responses to climate change that increase plant fitness under future predicted climates. The importance of such adaptive responses for population persistence will depend on the ability of plants to express evolved phenotypes in a community context, in the presence of other co-existing species. Furthermore, evolutionary change itself may alter the outcome of competition between co-existing species. Here we use a microcosm experiment to determine (i) whether climate-driven evolutionary changes in one species (*Festuca ovina*) are expressed in the presence of other co-existing species, (ii) whether evolutionary changes are adaptive under simulated climate change, and (iii) how such evolutionary changes may alter competitive interactions. We used F1 progeny of *F. ovina* plants collected from grassland plots exposed to experimental drought treatment and control conditions for 17 years at the Buxton Climate Change Impacts Laboratory (BCCIL). *Festuca ovina* individuals were planted in microcosms in which a single individual of three other species had also been planted. The microcosms were subjected to a two-month drought treatment mimicking the treatment applied at BCCIL. We measured *F. ovina* phenotypes and responses to drought and above-ground biomass production in all four species in each microcosm. Biomass and tiller growth responses differed between *F. ovina*

individuals with ancestry in control and drought environments at BCCIL. *Festuca ovina* plants with ancestry in the BCCIL drought treatment produced significantly less biomass than those with ancestry in the control environment. However, our results did not suggest that these changes increase plant fitness under simulated drought treatment. Finally, our results indicated a negative broad-sense genetic correlation between biomass production in *F. ovina* and the biomass production of neighbouring *Koeleria macrantha* individuals. Since evolution in response to drought has driven a reduction in biomass production in *F. ovina*, these results challenge the assumption that adaptation to climate change will necessarily lead to correlated increases in competitive ability.

5.2 Introduction

Plant populations across the globe are responding to changes in temperature and precipitation associated with climate change through shifts in species' geographical distributions (Kelly & Goulden 2008), epigenetic modifications (Rico *et al.* 2014; Nicotra *et al.* 2015), and plastic and evolutionary responses (reviewed by Franks, Weber & Aitken 2014). A growing number of studies are documenting climate-driven evolutionary changes, however, only a few have assessed the strength of these responses under ecologically realistic conditions, or determined whether evolutionary change is adaptive under the new climate (Franks, Weber & Aitken 2014). Evolutionary change can be maladaptive or neutral, and it is necessary to show a fitness benefit to establish that an adaptive evolutionary response has occurred (see Table 1 Merilä & Hendry 2014). Our

understanding of the potential for adaptive evolution to enable plant populations to persist through climate change is still very limited.

Increasingly there is a call for co-existing species to be incorporated into studies of adaptive responses to climate change (Brooker 2006; Gilman *et al.* 2010; Walther 2010; Northfield & Ives 2013; Alexander, Diez & Levine 2015; Crutsinger 2015; Farrer *et al.* 2015). A whole-community perspective is crucial for understanding the different aspects of plant responses to climate change. Firstly, plant-plant interactions can determine the detection, and magnitude of adaptation to climatic conditions (Bischoff *et al.* 2006; Brooker 2006; Liancourt & Tielbörger 2009; Ariza & Tielbörger 2011; Bocedi *et al.* 2013). In particular, competitive interactions between species can be of different relative importance dependent on the level of environmental stress (Grime 2001). In populations of *Bromus fasciculatus* and *Brachypodium distachyon,* locally adapted along an aridity gradient, Liancourt & Tielbörger (2009) found competition to be strong across the gradient, but competitive exclusion was only found in wetter habitats. Secondly, many studies have established the role of intraspecific trait variation in structuring communities (Turkington & Harper 1979; Aarssen & Turkington 1985; Fritz & Price 1988; Shevtsova *et al.* 1995; Booth & Grime 2003; Johnson, Lajeunesse & Agrawal 2006) and ecosystem function (Hughes *et al.* 2008; Hines *et al.* 2014; Schöb *et al.* 2015). Climatic selection on a trait, or suite of traits in one population may result in new phenotypes that change aspects of that species interaction with other species, which in turn may have cascade effects upwards on community structure or ecosystem function (Brooker 2006; Whitham *et al.* 2006; Whitlock 2014). Thirdly, modelling studies that aim to simulate eco-evolutionary responses to climate

change make the assumption that when populations are adapted to climate change this will result in higher growth rate and improved fitness (de Mazancourt, Johnson & Barraclough 2008; Johansson 2008; Norberg *et al.* 2012). However, there is little empirical data available with which to test whether these assumptions hold true in reality. If the evolutionary responses to climate change are compromised by other biotic or abiotic changes, then it may alter the ability of populations to persist through climate change. If co-existing species are excluded from studies of evolutionary responses to climate change we may fail to understand how adaptive evolutionary responses influence, and are influenced by, ecological interactions with co-existing species.

Although few studies have investigated adaptive evolution to current climate change, many have looked at the *local adaptation* of populations to their climatic conditions. Local adaptation is defined as the relative fitness advantage of a resident population in their local environment when compared with a non-resident population (Kawecki & Ebert 2004; Leimu & Fischer 2008). Studies of local adaptation are typically conducted by reciprocal transplant experiments, in which individuals from two (or more) populations are grown in the other environment(s), often along environmental clines. These types of studies can help us understand the potential for populations to adapt to altered climates and the relative importance of biotic interactions for facilitating, or hindering adaptive responses (Etterson 2004; Ramírez-Valiente *et al.* 2009; Ariza & Tielbörger 2011; Kim & Donohue 2013; Alexander, Diez & Levine 2015; Tomiolo, van der Putten & Tielbörger 2015).

The capacity for populations to evolve under climatic selection is principally determined by the level of genetic variation in traits, and the relationships between

traits (Etterson & Shaw 2001; Sgrò & Hoffmann 2004). The associations between traits can constrain their evolution such that fitness cannot be maximised in every trait, which results in trade-offs (Stearns 1992). Trade-offs underlie life-history strategy theory (Stearns 1992). The *C-S-R* model proposed by Grime (1974) is one model that aims to encapsulate many different aspects of trade-offs that define species-level adaptive life-history strategies: competitive, stress tolerant, and ruderal. Under drought conditions there are two main adaptive life-history strategies, *drought tolerance* which is the ability to survive under low water availability through physiological adaptation, and *drought escape*, which involves completion of the plant lifecycle before the onset of drought (Farooq *et al.* 2012). Drought tolerance falls under a stress-tolerant strategy within the C-S-R framework. Plants with drought escape strategies, on the other hand, have many of the traits associated with ruderal plant strategies under C-S-R life-history strategy theory. However, life-history strategy trade-offs imply that adaptation to the current climate may not always confer fitness under other conditions, in particular aspects of the biotic environment, such as competition from neighbouring plants, herbivory and pathogen resistance. For example, an intraspecific negative genetic correlation between resin content (which has a role in drought resistance and defence) and growth rate in *Diplacus aurantiacus* is likely to constrain evolutionary responses towards either a drought resistant and defensive strategy, or towards a fast-growing, competitive strategy (Han & Lincoln 1994). Where trade-offs constrain the relationships between traits important for adaptation to abiotic conditions and biotic interactions, we expect evolutionary responses to climate change to alter biotic interactions such as those involving competition.

Previous work at the Buxton Climate Change Impacts Lab (BCCIL) has provided evidence for climate-driven evolution in *F. ovina*. Ravenscroft, Whitlock & Fridley (2015) found significant genetic differentiation between plants from the drought and control plots at BCCIL using amplified fragment length polymorphism markers. In another experiment, in which clonal replicates of *F. ovina* collected from BCCIL were grown in a common environment, it was found that the population of *F. ovina* from the drought plots at BCCIL has diverged phenotypically from the control population (Whitlock, unpublished data). However, in Chapter 3, although we found heritable genetic variation in traits relevant to drought tolerance, we found no evidence of phenotypic differentiation between plants from different ancestral climates. These measurements were conducted in an ecologically simplistic environment, lacking competing species, and involving growth in cell trays. Thus, we now want to see whether key evolving phenotypes are expressed under more ecologically realistic conditions, and understand the extent to which evolved phenotypes are adaptive under drought, as well as understanding whether evolutionary changes may alter competitive interactions with other species.

In this chapter we use a microcosm community experiment, with a simulated drought treatment, to investigate adaptive responses to climate change and their ecological consequences. F1 progeny of *F. ovina* collected from a long-term climate change experiment at the Buxton Climate Change Impacts Laboratory (BCCIL) were grown in a microcosm containing three other plant species that are present in the BCCIL grassland community. Following 17 months of growth

outdoors in a common environment, microcosms were subjected to a two-month drought mimicking the drought treatment at BCCIL. The response and recovery of *F. ovina* to the drought treatment was measured and analysed as a function of their parent's ancestral climate at BCCIL. We also monitored the growth of neighbouring plants species. We ask (i) are evolutionary changes in phenotype in *F. ovina* expressed in the presence of co-existing species (ii) are these changes adaptive under simulated drought, and (iii) what are the consequences of evolutionary change for the growth of co-existing species?

5.3 Methods

5.3.1 Study system

At the Buxton Climate Change Impact Laboratory (BCCIL) a range of climate manipulation treatments have been imposed on 3 m × 3 m plots of calcareous species-rich grassland since 1994 (Grime *et al.* 2008). The treatments applied include summer drought, winter warming and supplementary summer rainfall, along with factorial combinations of these and non-manipulated control plots (full details in Chapter 1, Section 1.4). Plots are replicated five times in a randomised block design including control plots. Here, we focus on the drought treatment, which has seen the greatest quantity of species compositional change relative to control plots, in comparison with the other treatments applied at BCCIL. The drought treatment runs annually, through July and August. Automatic rain shelters are used to impose the drought, these cover the plot when it is raining, and return

to an off-plot position when the rain stops. This treatment has resulted in a significant reduction in the water potential in the surface soil at the end of the drought treatment (-1100 kPa in drought vs. -20 kPa in control plots, at 5 cm depth; see Figure 4 in Fridley *et al.* 2011).

5.3.2 Collection of F. ovina from BCCIL

In July 2010 living individuals of *F. ovina* were recovered from drought-treated and control plots at BCCIL by R. Whitlock, after 17 years of climate manipulation. Full details of the collection of material from BCCIL are provided in Chapter 2, Section 2.3.2. In brief, six individuals were collected per plot, resulting in the collection of 60 plants in total, 30 from drought-treated plots and 30 from control plots. Small bunches of 4–8 connected tillers were recovered from each plant sampled in the field which were subsequently established in 3 L pots in a 3:1 mix of John Innes No. 1 potting compost and medium grade Perlite (LBS Horticulture). Plants were housed in purpose-built raised "bays" at Ness Botanic Gardens, University of Liverpool, UK. They were maintained by seed head removal during the summer, biomass clipping in September and supplementary watering as required. Hereafter, this set of 59 clonal lines (one individual died following collection) is referred to as the *parent clonal library*[5].

During the flowering period of 2012 (May-June) the individuals in the parent clonal library were allowed to mate through natural wind pollination to create an F1 progeny array (full methods can be found in Chapter 2, Section 2.3.3). 58 of the

[5] Words in italics are defined in the glossary.

parent plants produced seed and 16 seeds from each maternal parent were selected at random and germinated. Of the seeds that germinated 8 seedlings per maternal parent were planted on and established. These individuals are maintained as clonal lines at Ness Botanic Garden, following the same regime as the parent clonal library, hereafter the *offspring clonal library*.

During the course of the microcosm community experiment we pedigreed the offspring clonal library. Full methods of the pedigree reconstruction can be found in Chapter 2, Section 2.5.3. In brief, plants from the *parent clonal library* and the offspring clonal library were genotyped at 9 microsatellite loci and the genetic data were used in a full probabilistic parentage analysis, carried out using MASTERBAYES (Hadfield, Richardson & Burke 2006) in the software package R (R Development Core Team 2008). Parentage inferences with a probability of greater than or equal to 0.5 were stored.

5.3.3 Microcosm experiment

5.3.3.1 Plant materials

In February 2014 we initiated a microcosm community experiment, in which plants were grown in a common environment for 17 months, followed by a simulated drought treatment (Figure 5.1). In the microcosm we grew four species that are abundant at BCCIL and common to calcareous grasslands: *F. ovina, Koeleria macrantha* (Ledeb.) Schult., *Carex caryophyllea* Latourr., and *Carex panicea* L.

Our focal species was the perennial grass *F. ovina*, for which plant material came from the *offspring clonal library*. A total of 48 clonal lines of *F. ovina* were

selected, spanning the known range of trait variation within the experimental population. The 48 clonal lines were selected based on their maternal parent's climate treatment plot and soil depth at BCCIL; full details of the method used to select the clonal lines are provided in Appendix 5, Section A5.1. Each microcosm contained a single clonal replicate of one of these clonal lines.

A single individual of each of three other species, *K. macrantha*, *C. caryophyllea*, and *C. panicea* was also planted in each microcosm (Figure 5.2). Material for *C. panicea* and *K. macrantha* was sourced from a clonal archive of plants that were collected from BCCIL in the same way as the *F. ovina* individuals in the *parent clonal library*. We used 12 different clonal lines of *K. macrantha* and 18 different clonal lines of *C. panicea*. The clonal lines of *K. macrantha* and *C. panicea* were selected such that we used equal numbers of plants originating from the drought and control treatments at BCCIL. We used a single clonal line of *C. caryophyllea* that had originally been collected from Cressbrookdale (clonal line Cc09; Whitlock *et al.* 2007).

The four species were selected to represent a range of responses to the drought treatment at BCCIL. *Festuca ovina* and *K. macrantha* are relatively drought tolerant; both have increased in abundance in the BCCIL drought treatment plots (Fridley *et al.* 2011). *C. panicea* is drought-sensitive, and has decreased in abundance under the drought treatment (Fridley *et al.* 2011). *C. caryophyllea* has changed little in abundance at BCCIL in response to the drought treatment (Fridley *et al.* 2011). However, the clonal line used in this study has a competitive growth form, and may be drought-sensitive (Whitlock, personal communication). Further

details of the responses of *F. ovina, K. macrantha, C. panicea* and *C. caryophyllea* to the drought treatment at BCCIL are provided in Table 5.1.

Table 5.1 Characteristics of the 4 species used in this experiment, their frequency and their responses to manipulated climate change as seen at BCCIL, data taken from Table 1 in Fridley *et al.,* (2011).

Species	**Growth form**	**Frequency***	**Effects of imposed drought†**
Festuca ovina	Grass	223	Positive
Koeleria macrantha	Grass	156	Positive
Carex panicea	Sedge	139	Negative
Carex caryophyllea	Sedge	273	No effect

* Frequency is the number of occurrences within 240 quadrats.

† A positive effect of imposed drought indicates higher abundance, and negative effect indicates lower abundance in the drought climate treatment plots at BCCIL compared to control plots.

5.3.3.2 Experimental design

The microcosm experiment was initiated during February 2014 and ran for 30 months (Figure 5.1). Each microcosm consisted of a 2 l plant pot filled with 80 mm of limestone chippings, covered by 1:1 mixture of natural redzina soil and perlite (LBS Horticulture) filled up to the top of the pot (total pot depth 180 mm). Each of the 48 clonal lines of *F. ovina* was replicated three times, in each of two different moisture-availability treatments (drought and control; one replicate of each treatment in each of three blocks), resulting in a total of 288 microcosms. Four tillers of *F. ovina* and *K. macrantha*, and a single ramet of each of *C. panicea* and *C. caryophyllea* were planted in each microcosm in the pattern shown in Figure 5.2. The plant material of the *Carex* species was standardised prior to planting by removing all rhizomatous tissue from each ramet. Replicate ramets and tillers of the 18 clonal lines of *C. panicea* and the 12 clonal lines of *K. macrantha* were

allocated to the microcosms such that all clonal lines were represented in each block and treatment group. On 08/02/2014 the leaf material of each plant in each microcosm was cut to a height of 25 mm above the surface of the soil to standardise biomass. We checked whether each plant in the microcosm experiment had established on 03/07/14. 38 individuals of *C. panicea* and 14 individuals of *C. caryophyllea* did not establish, and these were re-planted with fresh material on the 04/07/14.

We also created 36 additional "ambient" control microcosms. These were grown in identical conditions for the first 17 months of the experiment prior to the imposition of the drought treatment. Once the drought treatment was started, these microcosms were placed outside of the overhead canopy that was used to impose the drought treatment. Thus, these "ambient" controls could be used to test the effects of the drought shelter canopy on the microcosms. The ambient control microcosms were created using 12 of the clonal lines represented in the experiment, also replicated clonally three times.

Microcosms were kept outside in 3 purpose-built "bays", Figure 5.3. All of the microcosms were grown under a common garden environment for the first 17 months of the experiment, during which the plants established under the ambient conditions in Ness Gardens. During this phase of the experiment microcosms were supplemented with 200 ml of de-ionised water if rainfall was low for between 7 to 9 days. Regular weeding was carried out throughout the year. During the summer of 2014 (the first flowering season of the experiment) flower heads were removed from all plants that produced flowering tillers and counted. At the end of

September 2014 all above-ground plant material was cut to a height of 25 mm and the material was removed.

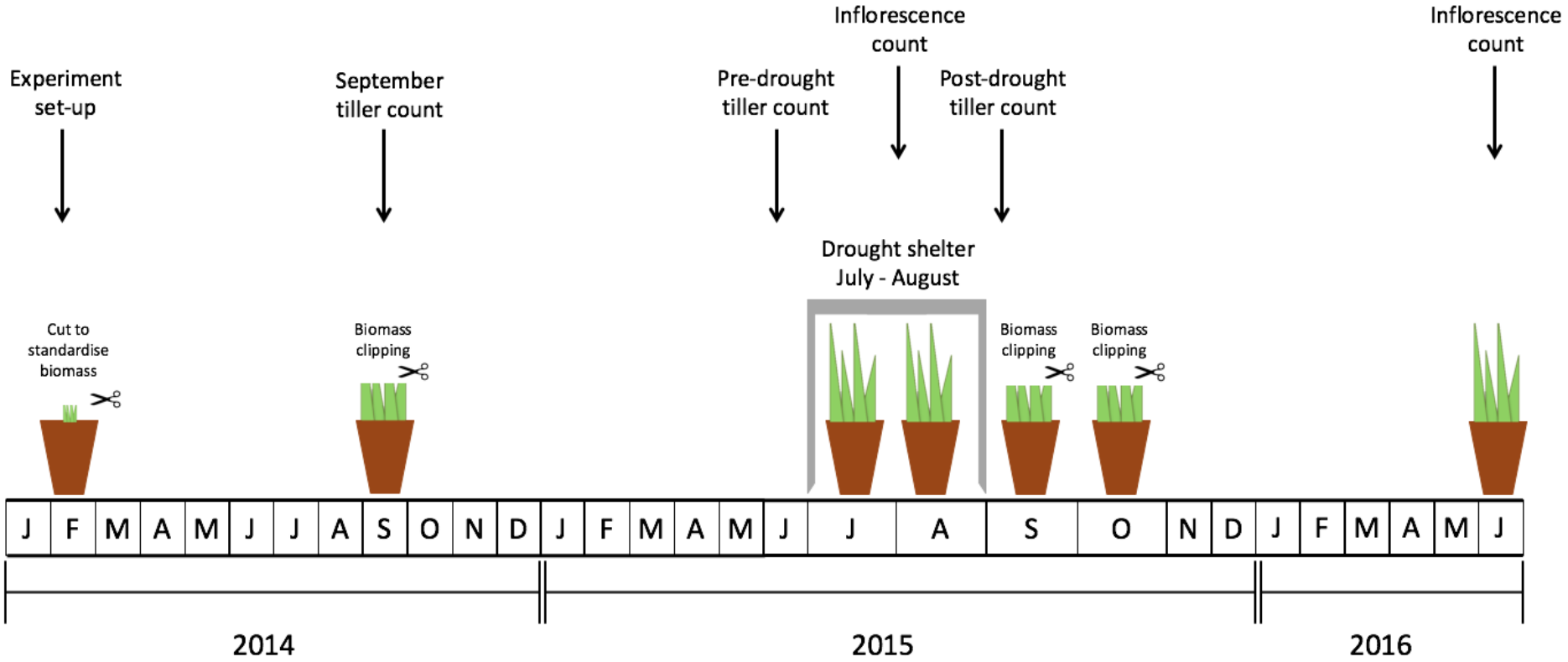

Figure 5.1 Timeline of the experiment, from initiation in February 2014 to completion in June 2016.

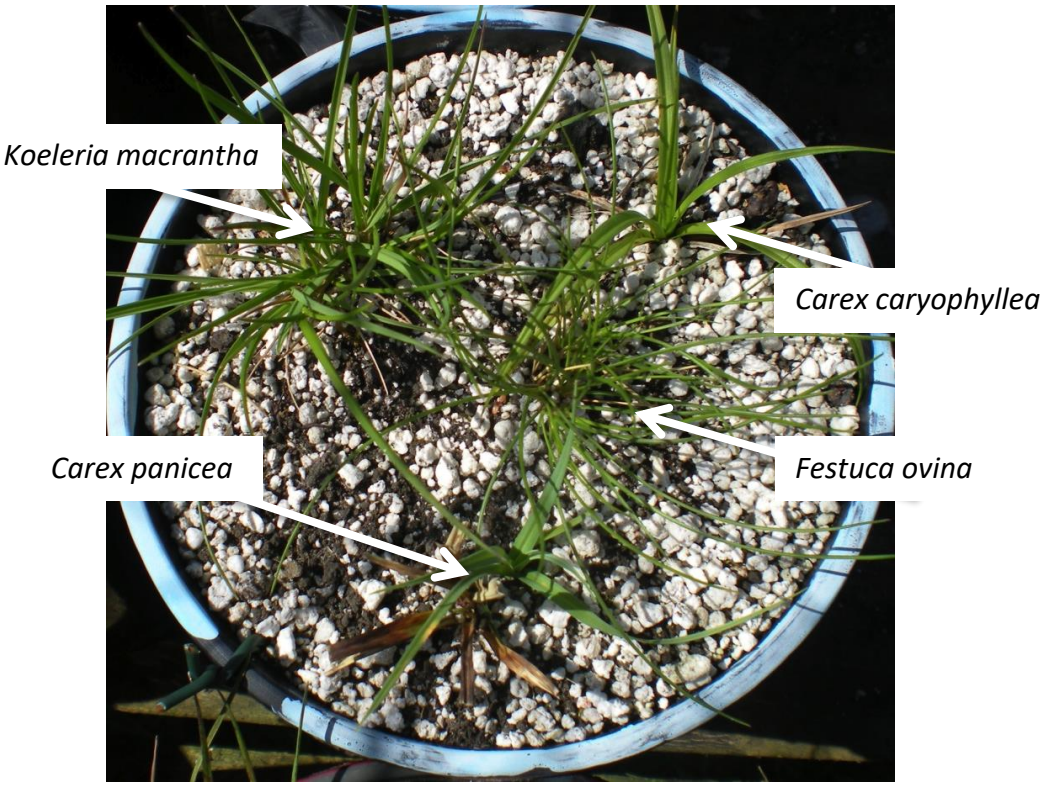

Figure 5.2 A picture of one of the experimental microcosms with the 4 species *F. ovina*, *C. panacea*, *C. caryophyllea*, *K. macrantha* labelled.

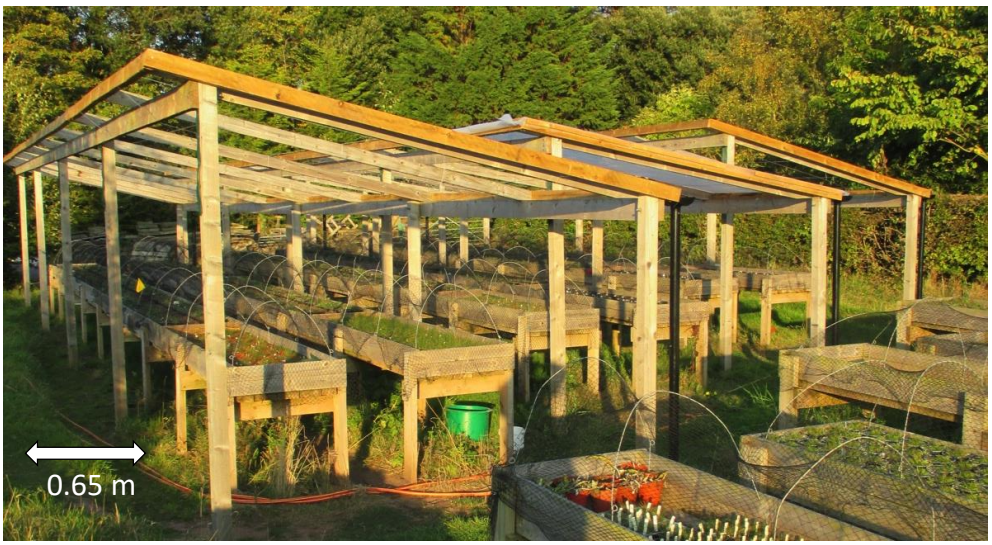

Figure 5.3 The purpose-built experiment bays where the experiment took place. The frame above the bays was used for attaching the polythene covering used to implement the drought during July and August 2015.

5.3.4 Drought manipulation

During July and August 2015 a drought was imposed on the microcosms by the placement of a canopy of clear polythene (125 µm, LSB Horticulture) over the bays. During this drought treatment, control microcosms were watered with 200 ml deionised water twice a week. Drought microcosms were watered to mimic levels of moisture availability in the drought treatment at BCCIL (Fridley *et al.* 2011). We monitored soil moisture content (method described below) and adjusted watering to replicate the moisture availability experienced by plants in the drought treatment at BCCIL. Drought microcosms received 100 ml of deionised water on the 30 July and the 10 and 20 of August. The ambient control microcosms were placed in a bay outside of the canopy to monitor the effect of the canopy itself on the experiment. Rainfall on the ambient microcosms was monitored and they were topped up with water twice a week so that soil moisture matched that in the control microcosms.

Soil moisture was monitored during the imposed drought treatment with a soil moisture probe (Theta Probe ML2x, Delta-T Devices Ltd). Soil moisture readings were taken in mV and then transformed to volumetric water content (details provided in Appendix 5, Section A5.2). Soil moisture in drought-treated microcosms fell to a minimum of 0.01 $Kg.L^{-1}$ on the 30/07/2015 and was then maintained at an average of 0.05 $Kg.L^{-1}$ for the month of August (Figure 5.4). Light flux measurements were taken at 10:00, 13:00 and 16:00 on three days during the drought treatment, both under the canopy and outside of the canopy, with a Solex SL-200 digital lux meter. Lux was converted to Photosynthetically Active Radiation (PAR) by multiplying by 0.018 $\mu mol.s^{-1}.lm^{-1}$ (the constant for clear daylight; Osram Sylvania

2005). Temperature and humidity were measured with Tiny Tag Plus 2 data loggers (Gemini data loggers, Sussex, UK). A breakdown of the differences in environmental conditions under the shelter and outside of the shelter can be found in Table 5.2.

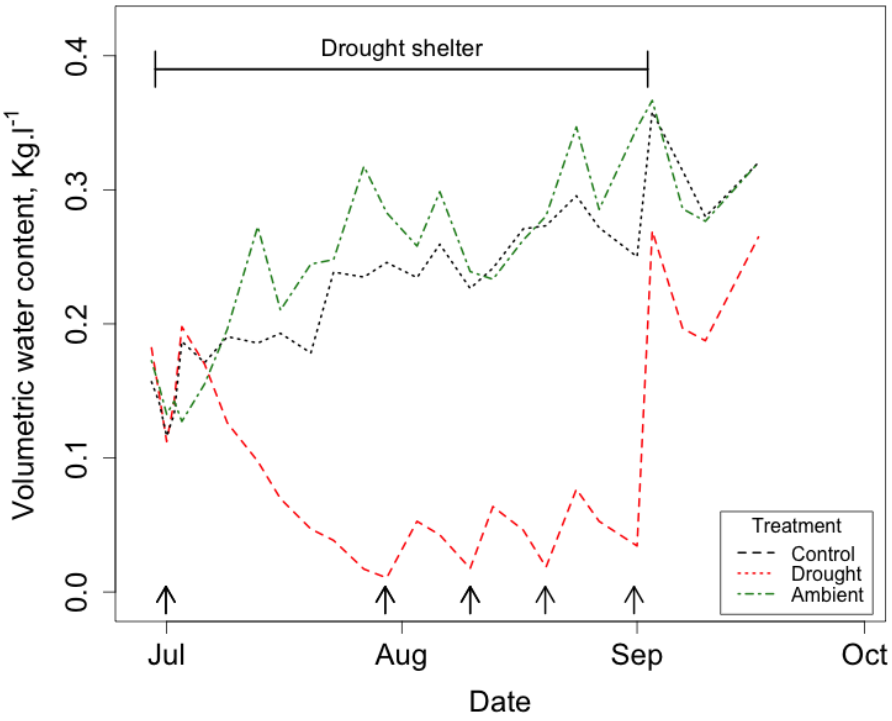

Figure 5.4 Soil moisture profile in the microcosms during the drought treatment. Arrows indicate watering in the drought microcosms. Control microcosms were watered twice weekly with 200ml deionised water. Ambient microcosms were kept outside of the drought canopy and watered to match moisture availability in the control microcosms.

Table 5.2 The mean, minimum and maximum temperatures and humidity during the course of the experimental treatment for each bay.

	Temperature (°C)			Humidity (%)	Light at 13:00 (PAR)		
	Mean	Min	Max	Mean	Mean	Min	Max
Bay 1	17.7	7.1	45.9	75.3	860.4	174.6	1526.4
Bay 2	17.4	7.3	45.0	76.9	579.6	192.6	1060.2
Bay 3	17.7	6.1	38.7	75.2	945.0	228.6	1618.2
Ambient bay	17.3	4.9	43.6	62.2	1306.8	349.2	2061.0

5.3.5 Trait data collection

Reproductive effort was measured through counts of the number of flowering tillers and seeds. Tiller counts were taken as a measure of asexual reproductive effort through clonal growth. We used above-ground dry biomass production over 25 mm above the soil surface as measurement of competitive ability. The correlation between the total vegetative biomass of an individual and the vegetative biomass 25 mm above the soil was strong, see Appendix 5, Section A5.3, Figure A5.1. We also measured the proportion of living canopy surface, along with other measures derived from this, to understand how senescence affects the response and recovery of the plant to drought. Full details of the methods used for trait data collection are provided in Table 5.3.

Table 5.3 Methods for measuring trait data and details of when measurements were taken.

Trait	Method	When	Species
Inflorescence number	The total number of flowering tillers (inflorescences) on an individual plant.	June-July 2015 June 2016*	*F. ovina*, *C. panicea,* *C. caryophyllea,* *K. macrantha*
Seed number	The total number of seeds for all inflorescences on an individual plant.	July 2015	*F. ovina*
Tiller number	The total number of tillers on a plant. A tiller was defined as the collection of blades of grass held within a single sheath (see Figure 977 in Stace 2010).	September 2014, June 2015, September 2015	*F. ovina*
Post drought biomass	The total vegetative biomass 25 mm above the soil surface was collected, dried for 1 week at 55 °C and weighed.	1-10th September 2015	*F. ovina*, *C. panicea,* *C. caryophyllea,* *K. macrantha*
October re-growth biomass proportion	One month after the post-drought biomass collection a second biomass clipping was taken to measure the re-growth of vegetative biomass. This biomass clipping was dried for 1 week at 55 °C and weighed. This was converted into a proportion of re-growth from the September biomass measurement, full details of this calculation are provided in Appendix 5, Section A5.4.	5th October 2015	*F. ovina*
Percentage of living canopy surface	We used photographs of the microcosms to monitor the senescence of the leaf canopy surface during the drought. Images were analysed using ImageJ (Abramoff, Magalhaes & Ram 2004)	9th July, 6th August, 1st September 2015	*F. ovina*

	to calculate the percentage of living, green plant leaf and stem tissue relative to total living and senesced plant tissue. Full details of this method are provided in Appendix 5, Section A5.5.		
Change in the percentage of living canopy surface	Difference between percentage of living canopy surface 1st of September and percentage of living canopy surface 9th July	NA	*F. ovina*
Change in the number of tillers	Difference between September 2015 tiller count and July 2015 tiller count	NA	*F. ovina*
The percentage of living canopy per tiller at the end of the drought	Percentage of living canopy surface from the 1st of September / September 2015 tiller count	NA	*F. ovina*
Change in the percentage of living canopy per tiller	(Percentage of living canopy surface from the 1st of September / September 2015 tiller count) – (percentage of living canopy from the 9th July / July 2015 tiller count)	NA	*F. ovina*

* In 2016 data on the inflorescence number was only collected for *F. ovina* and *K. macrantha*

5.4 Statistical analysis

5.4.1 The dataset

The reconstruction of the offspring clonal library pedigree took place after the initial planting of the microcosm experiment. Of the 48 clones initially planted in the experiment, the parentage analysis was able to predict the father of 46 clonal lines with a probability of no less than 0.5. The analyses presented here are restricted to these 46 clonal lines, which allowed the ancestral climate of both parents to be modelled in our analyses. The 46 clonal lines consisted of 12 clonal lines with pure control ancestry (both parents from control plots), 20 clonal lines with hybrid ancestry (one parent from each of a control and drought plot) and 14 clonal lines with pure drought ancestry (both parents from drought plots). Each clonal line was replicated three times in each climate treatment, resulting in data from a total of 276 microcosms being analysed.

To distinguish between the drought applied at BCCIL from the drought applied to the microcosm community experiment, we use the following terminology throughout this chapter: *ancestral climatic environment* refers to the climate treatment received by an individual plants parents at BCCIL, either *pure drought ancestry* (both parents from drought plots), *hybrid ancestry* (one parent from each of drought and control plot) or *pure control ancestry* (both parents from control plots). *Simulated drought treatment* refers to the drought applied to the microcosm community experiment in 2015.

5.4.2 Generalised linear mixed-effect model specifications

We used Generalised Linear Mixed-effects Models (GLMMs) to examine how *F. ovina* phenotypes were influenced by the plant's ancestral climatic environment and the drought treatment. All statistical analyses were carried out using R (R Development Core Team 2008) in the package MCMCGLMM (Hadfield 2010). MCMCGLMM uses a Bayesian approach to fit GLMMs; for a discussion of the Bayesian approach see Chapter 2, Section 2.5.2. Unless specified otherwise, models were run in MCMCGLMM for 1,300,000 iterations, with a burn in of 300,000 and a thinning interval of 1,000, resulting in a sample size of 1,000. Maternal clone identity, paternal clone identity and individual clone identity were fitted as sets of random effects. The prior for variance components was a non-informative uniform improper prior distribution on the standard deviation of the random effects (specified as $V = 1.0 \times 10^{-16}$, $nu = -1$ in MCMCGLMM), as recommended by Gelman, (2006). Sensitivity to starting parameters was tested in each model by running the model three times with over-dispersed chain starting values using the function *start=list(QUASI=FALSE)*, and using the Gelman-Rubin diagnostic to assess convergence (Gelman & Rubin 1992). The blocking factor for bay (3 levels) was fitted as a fixed effect in models, and contrasts for the bay variable were centred to allow estimation of the remaining parameters at a notional "average" bay. We used the *autocorr* function to check the autocorrelation, which is the level of non-independence between successive samples of the chain. The autocorrelation at lag 1 (i.e. between successive stored samples) was checked, and values below 0.15 were required for the model to be accepted, following the recommendation of Hadfield (2012).

In models containing pre-drought traits ancestral climate was fitted as a fixed effect with three levels (pure control, hybrid, and pure drought ancestry). In models containing during-drought and post-drought traits ancestral climate and the simulated drought treatment were fitted as fixed effects. Model reduction was carried out based on comparison of *pMCMC* values of the parameters of interest and, where appropriate, comparison of the Deviance Information Criterion (DIC). Post-hoc comparisons for particular parameter contrasts were made by re-levelling variables as needed. Parameters of interest were extracted as the mean of the posterior distribution for that parameter. We report "*95% credible intervals"* which define the range within which we expect, with a probability of 0.95, the true parameter value to be located. Credible intervals were calculated using the *HPDinterval* function in MCMCGLMM. Our traits included continuously distributed and count variables; each trait included in the analysis was modelled with an appropriate error distribution, preceded by data transformation where necessary (Table 5.4). Models were also fitted in LMER (Bates *et al.* 2015), where possible, to provide a comparison with the MCMCGLMM model. These models confirmed that parameter estimates were in the same direction and at the same magnitude as those of the MCMCGLMM model.

Table 5.4 Error distributions and transformations applied to trait data

Trait	Measured before, during or after the drought	Transformation	Error distribution
Number of tillers September 2014	Pre-drought	NA	Gaussian
Number of flowering tillers July 2015	Pre-drought	NA	Gaussian
Total number of seeds	Pre-drought	NA	Poisson
Average number of seeds per flowering tiller	Pre-drought	NA	Gaussian
Percentage of living canopy surface	During-drought	NA	Gaussian
Change in the percentage of living canopy surface from the start to the end of drought treatment	During-drought	NA	Gaussian
Change in the number of tillers from the start to the end of drought treatment	During-drought	NA	Gaussian
Percentage of living canopy surface per tiller at the end of the drought treatment	During-drought	Log	Gaussian
Change in the percentage of living canopy surface per tiller from the start to the end of drought treatment	During-drought	NA	Gaussian
F. ovina post drought biomass	Post-drought	Square root	Gaussian
F. ovina October re-growth biomass proportion	Post-drought	Square root	Gaussian
Number of flowering tillers June 2016	Post-drought	NA	Poisson
K. macrantha biomass	Post-drought	Square root	Gaussian
C. panicea biomass	Post-drought	NA	Exponential
C. caryophyllea biomass	Post-drought	NA	Exponential

5.4.3 Pre-drought evolutionary responses under a common environment

To test whether climatic selection at BCCIL has resulted in the evolution of phenotypes of *F. ovina,* we analysed the number of tillers in September 2014, the number of flowering tillers in July 2015, the total number of seeds in 2015 and the average number of seeds per flowering tiller in 2015 in a GLMM to test for phenotypic differentiation based on ancestral climate at BCCIL. The ancestral climate at BCCIL was fitted as a fixed effect with 3 levels (pure control, hybrid and pure drought ancestry). Reported *pMCMC* values are for the comparison between plants from pure control ancestry and pure drought ancestry.

Flowering tillers develop from vegetative tillers that were initiated in the preceding growing season, and vernalised in the preceding winter. Thus we expected there to be a relationship between the number of vegetative tillers in September 2014, and the number of flowering tillers in the subsequent flowering season (July 2015). To examine whether evolutionary responses in asexual reproduction (tiller number) could result in altered sexual reproduction (number of flowering tillers), we analysed the number of vegetative tillers a plant had in September 2014 and the number of flowering tillers it then produced in July 2015 with a multi-response GLMM. We used a multi-response model to allow us to control for variation in both the predictor and response variable simultaneously, and to allow both within and between clone variance to be estimated. Bay was fitted as a fixed effect and clone was fitted as a random effect. The correlation coefficient was calculated at the within and between clone level using the *posterior.cor* function in MCMCGLMM.

5.4.4 Phenotypic responses under simulated drought treatment

To assess how *F. ovina* grew through the simulated microcosm drought we analysed the percentage of living canopy surface data with two complementary analyses. First, we used a GLMM to analyse the data from all three data collection dates (9th July, 6th August, 1st September 2015). The microcosm drought treatment and the collection date were fitted as fixed effects. Microcosm number was added as an extra random effect to control for the dependency of repeated observations from the same microcosm. Second, the data for each collection date were analysed separately in a GLMM with the microcosm drought treatment as a fixed effect.

If plant phenotypes have evolved to be adaptive under drought conditions then we expect to observe plants with drought ancestry either having greater fitness through the drought, or recovering more quickly following the drought, compared to plants of control ancestry. One way plants may cope with drought is through the way they manage the senescence of leaf tissue at the tiller level. To assess how tiller dynamics and leaf senescence responses may contribute to adaptation to drought, we applied GLMM to the change in the percentage of living canopy surface from the start to the end of the drought, the change in the number of tillers from the start to the end of the drought, the percentage of living canopy surface per tiller at the end of the drought, and the change in the percentage of living canopy surface per tiller from the start to the end of the drought. To test whether evolved traits are adaptive in a plant's resistance to drought, and recovery from drought, we analysed the vegetative biomass at the end of the drought and the proportion of biomass re-growth a month following the drought with a GLMM.

To test whether adaptive responses to drought are expressed through altered reproductive output in the year following a drought in *F. ovina,* we analysed the number of flowering tillers in June 2016 in a GLMM. In each model the microcosm drought treatment and the ancestral climate were fitted as fixed effects.

5.4.5 Consequences of evolutionary change for species interactions

We used biomass as a measure of individual performance in focal *F. ovina* individuals and in neighbouring individuals of the other three plants in each microcosm. We used bivariate response models to estimate total, genotypic and residual (plastic) correlations between biomass production in *F. ovina* and biomass production in each of the other species. Use of these models has two main advantages. First it allowed us to control for variation in both the predictor and response variable simultaneously. Second it allowed among-clone variance (and covariance) in phenotype to be estimated within (and among) the response variables. From the estimated variance and covariance components, we then estimated the broad-sense genetic covariance (genotypic covariance) between the biomass of *F. ovina* and the biomass of the co-existing species (fitted as the second response variable in each model). This measure of genetic covariance is akin to a "broad-sense" genetic correlation and represents an *inter-specific indirect genetic effect, sensu* Whitham *et al.* (2006), where the genes in one individual influence the phenotype of another individual.

The dataset consisted of the biomass measurements from 274 microcosms. Two microcosms were identified as outliers and excluded from the analysis because

they had excess vegetative biomass growth. Appendix 5, Section A5.6 provides a justification for the exclusion of these microcosms. During the running of the experiment 11 individuals of *C. panicea* died (7 during establishment, 4 during the drought) and 5 individuals of *C. caryophyllea* died (2 during establishment, 3 during the drought). Microcosms containing these individuals were excluded from the analysis of *C. panicea* and *C. caryophyllea*, leaving a total of 263, and 269 microcosms for each analysis respectively.

In each model the clone identities of *F. ovina* and those of the co-existing species were fitted as random effects. The prior used was an inverse Wishart distribution (V = 1, nu = 0.002). Only a single clone of *C. caryophyllea* was used in the experiment, so only the clone identities of *F. ovina* could be fitted as random effects in the model with *C. caryophyllea*. The correlation coefficient and its credible intervals was calculated at the among-clone level and the residual level using the *posterior.cor* function in MCMCGLMM.

5.5 Results

5.5.1 Environmental conditions and drought manipulation

The environmental conditions under the drought shelter varied from the environmental conditions measured outside the shelter (in the ambient bay). Temperatures ranged between 6.1°C and 45.9 °C under the shelter, compared to 4.9 °C to 43.6 in the ambient bay. Humidity was, on average, 21.9% higher under the shelter (75.8%) compared to outside the shelter (62.2%). Light was reduced

under the shelter, ranging between 174.6 PAR to 1618.2 PAR, compared to a range of 349.2 PAR to 2061.0 PAR outside the shelter. Plant growth also differed under the shelter, with an average plant biomass for *F. ovina* of 145.1 mg under the shelter compared to 85.9 mg outside the shelter in the ambient bay.

5.5.2 (i) Are evolutionary responses expressed in the presence of co-existing species in a common environment?

In measurements taken before the drought treatment, plants with pure drought ancestry produced fewer tillers than plants of pure control ancestry (*pMCMC* = 0.086). Pure drought ancestry plants produced on average 14.9% fewer tillers than control ancestry plants (Figure 5.5 **A**). Neither a plant's number of flowering tillers in July 2015 (Figure 5.5 **B**), its average number of seeds per flowering tiller (Figure 5.5 **C**), nor its total number of seeds were predicted by its ancestral climate at BCCIL, (*pMCMC* = 0.284, *pMCMC* = 0.384 and *pMCMC* = 0.506, respectively).

The number of flowering tillers was strongly positively correlated with the number of vegetative tillers in the September prior to flowering (bivariate response model; *pMCMC* < 0.001; Figure 5.5 **D**). The broad-sense genetic correlation between these traits (among clone correlation) was *r* = 0.805 (credible interval 0.541–0.897), and the residual correlation was *r* = 0.680 (credible interval 0.565–0.719).

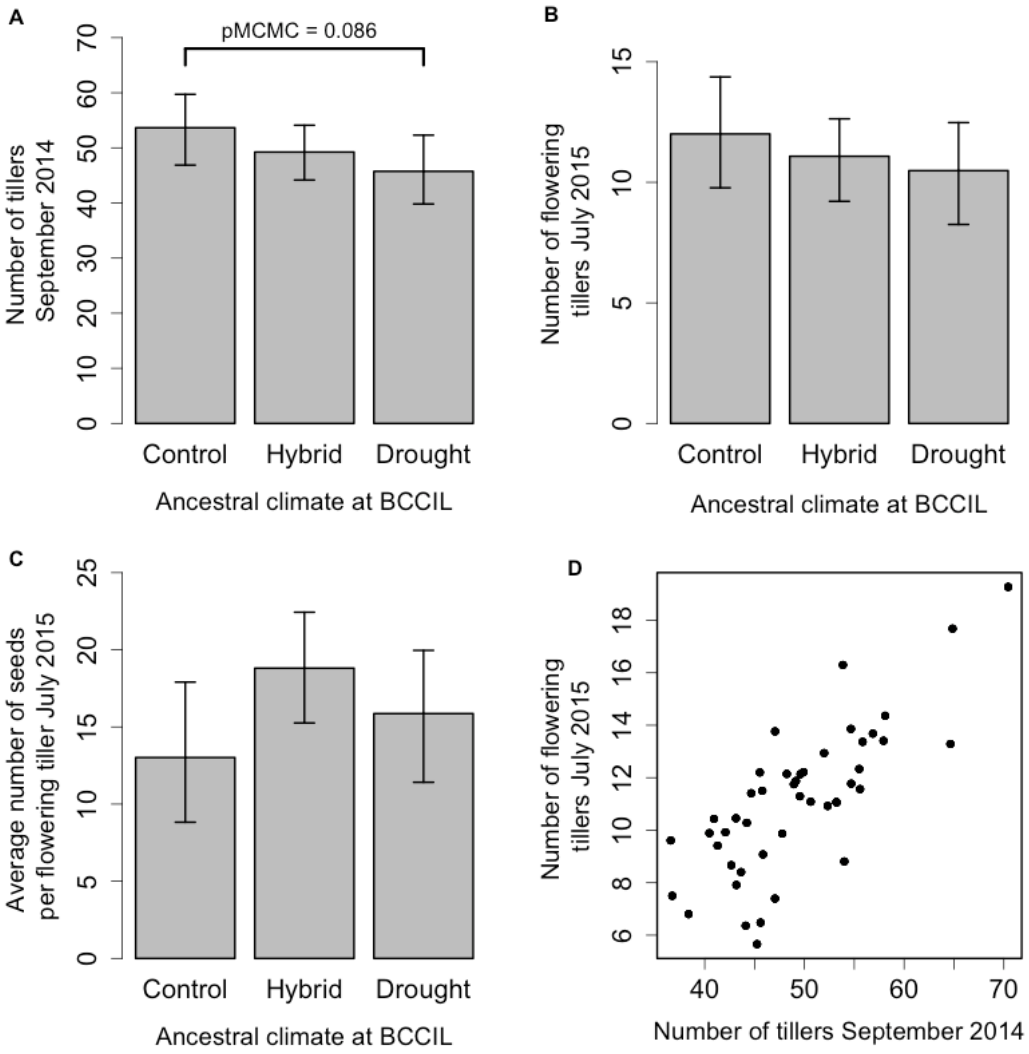

Figure 5.5 Phenotypic differentiation between sub-populations of *F. ovina* with ancestry in different climate treatments at BCCIL. **A)** The number of tillers in September 2014 (after 7 months of growth). **B)** The number of flowering tillers in July 2015. **C)** The average number of seeds per flower. Error bars represent 95% credible intervals. **D)** The number of flowering tillers in July 2015 plotted against the number of vegetative tillers in September 2014. Each point represents the MCMCGLMM model prediction for a single clone of *F. ovina*, extracted from the bivariate response model. This was re-run without bay fitted as a fixed effect to allow a single point to be fitted for each clone, and marginalised for each clone of *F. ovina*.

5.5.3 (ii) Are evolutionary responses adaptive under simulated drought?

Clones of *F. ovina* differed widely in the proportion of living canopy surface prior to the start of the simulated drought treatment, ranging from 1.7% to 84.6% (Figure 5.6).

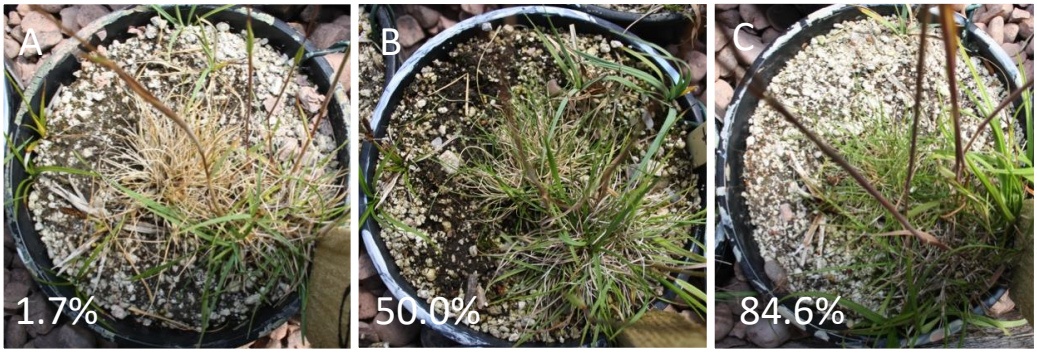

Figure 5.6 Examples of the total living canopy surface at the start of the simulated drought treatment.

On average, plants continued to grow through the drought treatment, i.e. they increased the percentage of living canopy surface. The percentage of living canopy surface changed over the course of the experiment depending on whether the plants were drought-treated or not (Figure 5.7). At the beginning of the drought treatment there was no significant difference in the percentage of living canopy surface area between plants in the drought and control treatment. By the middle and the final time points there were significant differences in the percentage of living canopy surface between drought-treated and control microcosms (*pMCMC* < 0.001). Plants from the control treated microcosms increased their percentage of living canopy surface area throughout the two-months of the drought treatment.

Growth in drought-treated microcosms occurred predominantly in the latter half of the treatment.

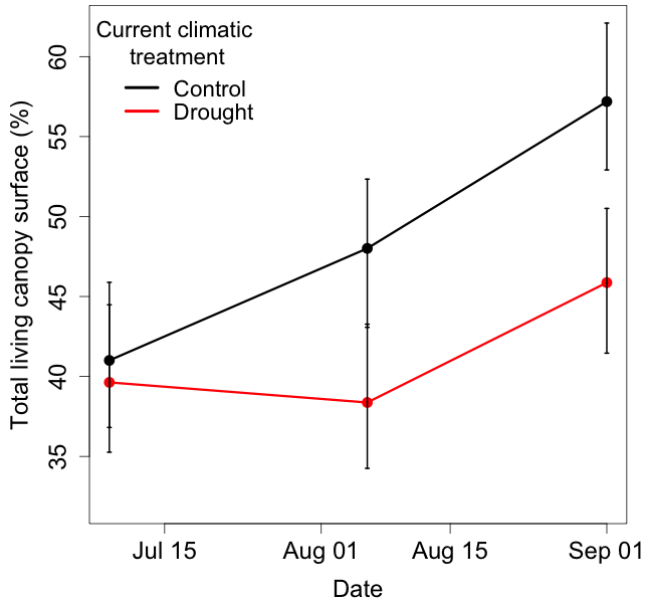

Figure 5.7 The percentage of living canopy surface area of *F. ovina* through the two-month simulated drought treatment. Error bars show 95% credible intervals.

Plants from drought and control ancestries at BCCIL did not differ in their percentage of living canopy surface, across two-months, when under the control conditions in the experiment (*pMCMC* = 0.702; Figure 5.8 **A**). Drought treatment induced decreases in percentage of living canopy surface area in plants of pure control ancestry and plants of pure drought ancestry (pure control ancestry *pMCMC* = 0.002; pure drought ancestry *pMCMC* < 0.001; Figure 5.8 **A**). Plants of pure control ancestry had on average a 17 % increase in the percentage of living canopy surface under control conditions over two-months, compared to a 9 % increase under drought conditions. Plants of pure drought ancestry had on average a 16 % increase in the percentage of living canopy surface under control conditions, compared with only a 4 % increase under drought conditions.

There was a net increase in the number of tillers from July 2015 to September 2015, although the change in the number of tillers for any individual *F. ovina* plant ranged from a gain of 28, to a loss of 25 tillers. Under control conditions plants of pure drought and pure control ancestry differed in growth increment (*pMCMC* = 0.042 Figure 5.8 **B**). Plants of pure control ancestry grew on average by 6.6 tillers over the two-month period in control conditions, whereas plants of pure drought ancestry grew on average by 3.2 tillers. Plants with pure control ancestry differed significantly in tiller growth between the drought treatment and control conditions (*pMCMC* < 0.001; average increase in tiller number = 6.6, control conditions versus 2.7 tillers, drought treatment; Figure 5.8 **B**). However, plants of pure drought ancestry did not experience a reduced rate of tiller growth under the drought treatment relative to control conditions (*pMCMC* = 0.752; Figure 5.8 **B**).

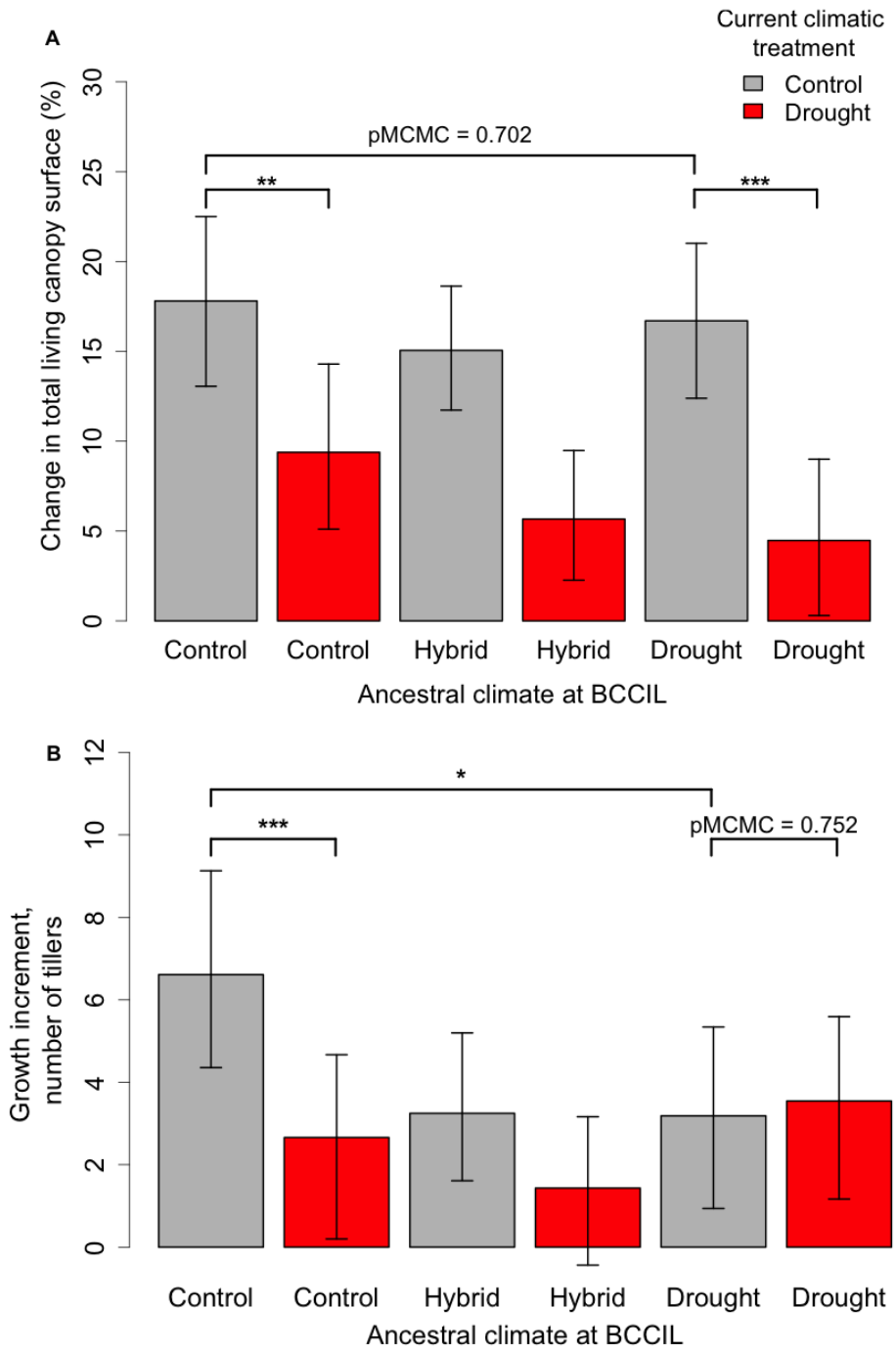

Figure 5.8 Differential responses of drought and control ancestry populations of *F. ovina* to two months' simulated drought treatment **A** Growth through drought (as the change in the percentage of living canopy surface) from July 2015 to September 2015, and **B** Growth increment (as the absolute change in the number of tillers) from July 2015 to September 2015, based on current climatic treatment and climate ancestry at BCCIL. For both plots: Control = both parents from control plots; hybrid = one parent from each of drought and control plot; drought = both parents from drought plots. Error bars show 95% credible intervals. Significance levels: * *pMCMC* < 0.05; ** *pMCMC* < 0.01; *** *pMCMC* < 0.001.

The drought treatment drove significant decreases in the percentage of living canopy surface per tiller—representing the tiller-level senescence response—in both plants of pure control ancestry and plants of pure drought ancestry (pure control ancestry: *pMCMC* = 0.010; pure drought ancestry: *pMCMC* = 0.002; Figure 5.9 **A**). Plants of pure control ancestry had, on average, a percentage living canopy surface per tiller of 1.25 under control conditions, compared to 1.05 under drought conditions. Plants of pure drought ancestry had, on average, a percentage of living canopy surface per tiller of 1.32 under control conditions, compared to 1.05 under drought conditions.

The drought treatment elicited significant reductions in the change in the percentage of living canopy surface per tiller from the start to the end of the drought treatment in both plants of pure control ancestry and plants of pure drought ancestry (pure control ancestry *pMCMC* < 0.038; pure drought ancestry *pMCMC* < 0.001; Figure 5.9 **B**). For this measure, a positive number means that the proportion of living tissue grew at a faster rate than number of tillers, while a value of 0 means that the rate at which the proportion of living tissue changed is equal to the rate at which tillers changed. Plants of pure control ancestry had, on average, a change in the percentage living canopy surface per tiller of 0.28 under control conditions, compared with 0.14 under the drought treatment. Plants of pure drought ancestry had on average a change in the percentage of living canopy surface per tiller of 0.35, compared to 0.06 under drought conditions.

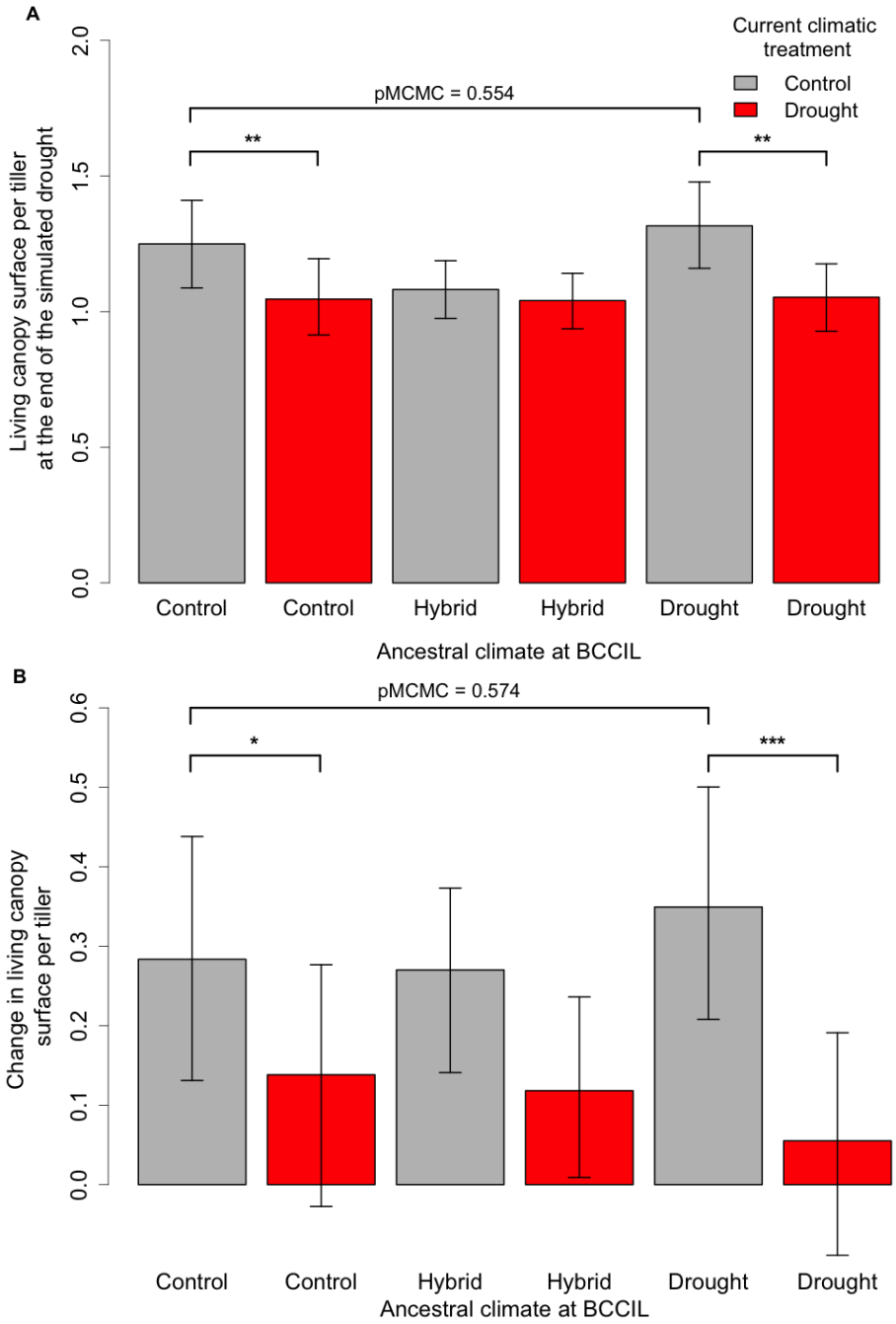

Figure 5.9 Plant management of senescence of leaf tissue at the tiller level in response to two months' simulated drought treatment. **A** The percentage of living canopy surface per tiller at the end of the simulated drought treatment and **B** The change in percentage of living canopy surface per tiller from July 2015 to September 2015, based on current climatic treatment and climate ancestry at BCCIL. Control = both parents from control plots; hybrid = one parent from each of drought and control plot; drought = both parents from drought plots. Error bars show 95% credible intervals. Significance levels: * *pMCMC* < 0.05; ** *pMCMC* < 0.01; *** *pMCMC* < 0.001.

Festuca ovina clones with pure control ancestry and those with pure drought ancestry differed significantly in biomass production (*pMCMC* = 0.012 Figure 5.10). Plants of pure drought ancestry had less vegetative biomass, on average 116.3 mg, while those with pure control ancestry, produced on average 181.3 mg of dry biomass.

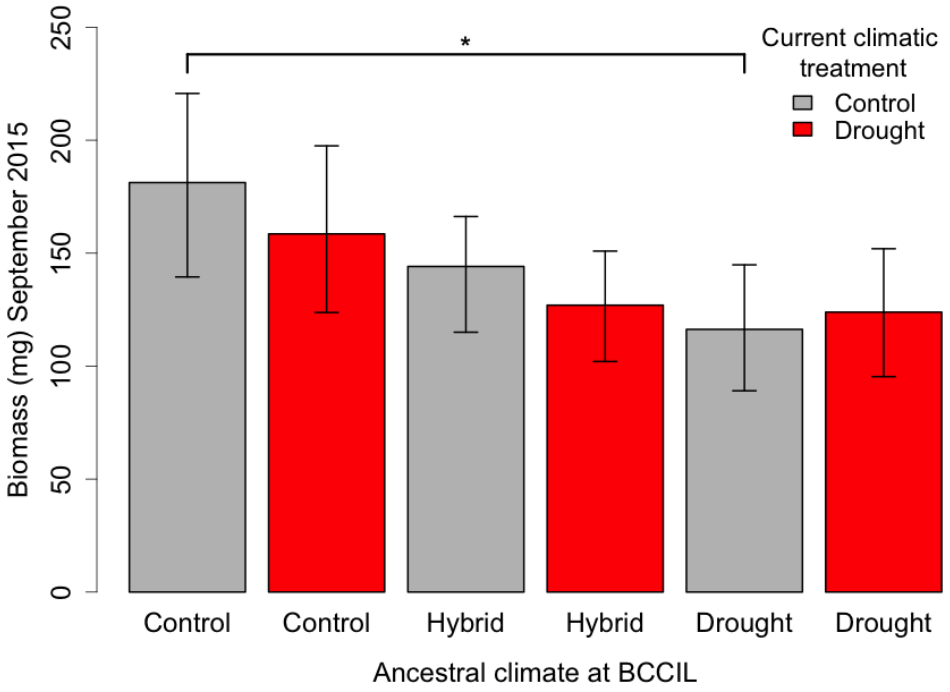

Figure 5.10 The vegetative biomass of *F. ovina* following one year's growth since the previous clipping. The drought treatment took place during the two months prior to the clipping. Control = both parents from control plots; Hybrid = one parent from each of drought and control plot; Drought = both parents from drought plots. Error bars show 95% credible intervals. * *pMCMC* < 0.05

Following the drought treatment, drought-treated and control plants differed significantly in the re-growth of biomass (*pMCMC* < 0.001). In the month following the simulated drought treatment plants that had been in the drought

treatment had greater re-growth than plants that had been in the control treatment (Figure 5.11), with an average of 2.7% regrowth compared to 3.7% regrowth for plants under the control and drought treatments, respectively. Plants with drought and control ancestries did not differ in the relative extent of biomass change following the simulated drought treatment (*pMCMC* = 0.912).

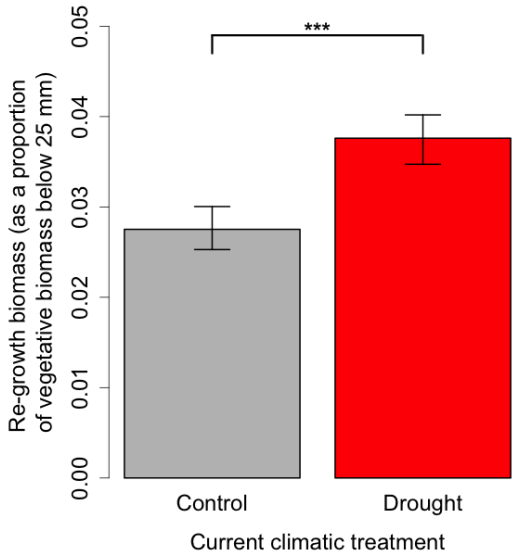

Figure 5.11 The re-growth vegetative biomass above 25 mm, as a proportion of the vegetative biomass below 25 mm. Error bars represent 95% credible intervals. *** *pMCMC* < 0.001

In the 2016 flowering season (the flowering season that followed the simulated microcosm drought), on average, *F. ovina* produced 7.1 fewer flowering tillers compared to 2015. However, there was no evidence that plants that had been under the simulated microcosm drought differed in the number of flowering tillers they produced, compared to plants that had been under control conditions (*pMCMC* = 0.422). Neither was their evidence that plants of drought or control ancestries differed in the number of flowering tillers that they produced in 2016 (*pMCMC* = 0.992).

5.5.4 *(iii) What are the consequences of evolutionary change for co-existing species?*

The average total biomass above 25 mm in a microcosm was 322.3 mg. *F. ovina* and *K. macrantha* both contributed almost equally to this, accounting for on average 45.6 % and 45.3 % of the vegetative biomass above 25 mm respectively, with *C. panicea and C. caryophyllea* accounting for only a small proportion of the canopy biomass (summary statistics provided in Table 5.5).

Table 5.5 Summary statistics of the vegetative biomass of each species in the microcosm and their contribution to the vegetative biomass above 25 mm.

Species	N	Mean (mg)	Range (mg)	Percentage contribution to total vegetative biomass above 25 mm in the microcosm (%)*
F. ovina	274	146.5	14.0 – 398.8	45.6
K. macrantha	274	146.7	16.0 – 480.5	45.3
C. panicea	263	16.4	0 – 68.2	3.9
C. caryophyllea	269	12.3	0 – 103.9	5.2

* Values based on 259 microcosms in which no individuals, of any species, died. A value of the percentage of biomass above 25 mm that each species contributed to an individual microcosm was calculated, and then averaged across all microcosms. Data reflect only the proportion of biomass above 25 mm not total vegetative biomass.

Vegetative biomass production in *F. ovina* showed a strong negative genotypic correlation with that in *K. macrantha* (bivariate response model Table 5.6; Figure 5.12 **A**), indicating that genetic variation for productivity in *F. ovina* drove phenotypic change in neighbouring *K. macrantha* plants. Similarly, vegetative biomass production in *K. macrantha* showed a strong and significant negative genetic correlation with the vegetative biomass production in *F. ovina*. These correlations are analogous to broad-sense genetic correlations between species,

and quantify the extent to which the phenotypic performance of one species is explained by genes residing in another. The vegetative biomass of *F. ovina* did not show correlation with the vegetative biomass production in either *C. panicea* or *C. caryophyllea* (Table 5.6; Figure 5.12 **B** & **C**).

Table 5.6 The correlation coefficients between *F. ovina* and each of the other co-existing species in the microcosms estimated using bivariate response models. The phenotypic correlation between the biomass of *F. ovina* and the biomass of the co-existing species is partitioned into that attributable to *F. ovina* clonal genotype, the co-existing species clonal genotype, or residual variance. The among-clone correlations are analogous to broad-sense genetic correlations between species, and describe the extent to which the phenotypic performance of one species is explained by genes residing in another. CI† = credible interval. Credible intervals that do not include 0 were considered statistically significant.

Biomass response variables (species pair)	Components of correlation	*r*	Lower CI†	Upper CI†
F. ovina* & *K. macrantha	Among *F. ovina* clone correlation	–0.537	–0.834	0.081
	Among *K. macrantha* clone correlation	–0.811	–0.966	–0.528
	Residual correlation	–0.183	–0.308	–0.060
F. ovina* & *C. panicea	Among *F. ovina* clone correlation	0.194	–0.379	0.614
	Among *C. panicea* clone correlation	0.252	–0.433	0.667
	Residual correlation	0.066	–0.392	0.499
F. ovina* & *C. caryophyllea*	Among *F. ovina* clone correlation	0.137	–0.391	0.595
	Residual correlation	0.122	–0.187	0.559

* We used only a single clone of *C. caryophyllea* so no variance components or corresponding correlations could be estimated for this species.

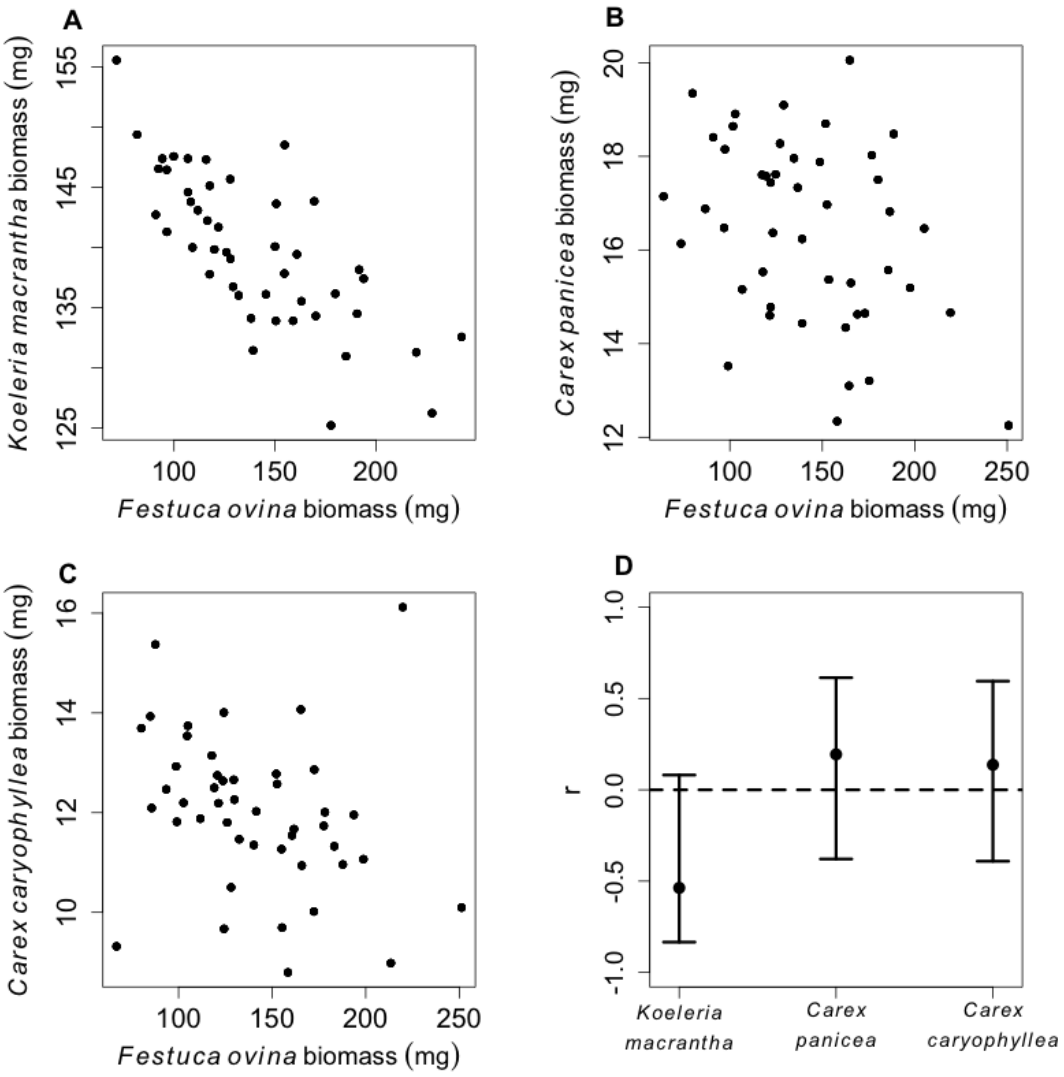

Figure 5.12 Relationship between biomass production in *F. ovina* and biomass production of each of three neighbouring plant species within experimental microcosms **A**) *K. macrantha* **B)** *C. caryophyllea* and **C)** *C. panicea*. Each point represents the MCMCGLMM model prediction for a single clone of *F. ovina*, extracted from the bivariate response model. The model was re-run without bay fitted as a fixed effect to allow a single point to be predicted for each clone. **D)** Point estimates and credible intervals for the among *F. ovina* clone genetic correlation in biomass phenotypes. Error bars show upper and lower limits of the 95% credible interval for each point estimate. Credible intervals that do not include 0 were considered statistically significant.

5.6 Discussion

In this chapter we used a microcosm experiment, combined with a drought treatment, to determine whether evolutionary changes in phenotype in *F. ovina* could be expressed in the presence of co-existing species, whether these responses are adaptive under a simulated drought and the consequence of these evolutionary responses for the growth of co-existing species. We have documented the evolution of a slower tiller growth rate and reduced vegetative biomass in response to long-term simulated drought in *F. ovina* at BCCIL. We did not find evidence that the evolution of these traits is adaptive under a short-term drought. We also found that there is a negative (broad-sense) genetic correlation between the biomass of *F. ovina* and the biomass of neighbouring *K. macrantha*. Given the evolution of reduced vegetative biomass in *F. ovina* under long-term drought, and the negative correlation that we find between the biomass of *F. ovina* and *K. macrantha*, our results suggest that evolutionary responses to climate change may alter competitive interactions among co-existing species, providing competitive opportunities for some species, while reducing the competitive ability of others.

5.6.1 Environmental conditions and drought manipulation

In this chapter we used a simulated drought to study the adaptive evolutionary responses of *F. ovina* to drought. The aim of the simulated drought was to impose conditions closely mimicking the drought treatment applied at BCCIL, whilst maintaining an experimental set-up in which numerous fine-scale measurements could be collected on individual plants of known ancestral climate

and parentage. The drought that we imposed was successful in keeping soil water content comparably in line with that measured through the drought at BCCIL (Fridley *et al.* 2011). However, the use of shelters altered other environmental conditions as compared to the ambient environmental conditions. On average, maximum and minimum temperatures were higher and humidity was greater under the shelter, while light was reduced, compared to outside the shelter. The growth of *F. ovina* was also different under the shelters, with plants producing on average 59.2 mg more vegetative biomass in pots under the shelters than those in 'ambient' pots (although different clonal lines of *F. ovina* were grown under ambient conditions, compared to those grown under the shelter, so the results are not directly comparable). The environmental differences experienced under the shelter compared to outside the shelter, and the potential resulting effect on plant growth, reduces the utility of these results for comparison with ambient conditions.

5.6.2 (i) Are evolutionary responses expressed in the presence of co-existing species in a common environment?

In this study we have documented the evolution of a significantly reduced biomass production in response to a long-term drought treatment. In general, studies that have experimentally manipulated precipitation to mimic predicted patterns of future climates have found that biomass production is reduced under decreased precipitation (Wu *et al.* 2011; Unger & Jongen 2014). However, here we have demonstrated that, in *F. ovina*, a reduction in biomass has evolved under the drought treatment, and is not simply the result of adverse conditions under the

drought treatment. We have also documented that tiller growth rate has evolved in *F. ovina*. Measuring the tiller growth rate during two months under a common environment, we find that plants of pure control ancestry produce around twice as many tillers as those of pure drought ancestry. These two traits, biomass production and tiller growth rate, are both important traits for ecosystem functioning and productivity. *Festuca ovina* is the dominant grass within the BCCIL grassland, therefore, the evolution of reduced biomass and tiller growth rate, has the potential to result in reduced productivity of the grassland ecosystem as a whole.

There is also a trend for a reduction in the number of flowering tillers in plants of pure drought ancestry, potentially arising from evolutionary changes in tiller growth. The number of flowering tillers is strongly correlated with the number of tillers in the preceding autumn. Tillers require vernalisation to have the potential for floral initiation. A higher tiller growth rate in plants of control ancestry results in more tillers available for vernalisation in the preceding autumn, and therefore more tillers are available to become flowering tillers in the following summer. This suggests that evolutionary changes to tiller number could affect aspects of reproductive output.

The direction of climatic selection on the 2015 measures of number of flowering tillers that we find here is consistent with the pattern found in the *parent microcosm experiment*. In the parent microcosm experiment, replicated clones of plants from the *parent clonal library* have been grown in a common garden, and it has been found that plants from the drought plots at BCCIL had a significant reduction in the number of flowering tillers compared to plants from the control

plots. Our results are also consistent with the pattern seen in the measurements taken on the plants of the *parent clonal library* discussed in Chapter 2, in which we also found a reduction in the number of flowering tillers in plants from drought plots at BCCIL. No measures of tiller growth rate or tiller number were taken in either of the studies described above, so we are unable to make direct comparisons about the consistency of differences in tiller growth rate between our study and the results from the *parent microcosm experiment* or the experiment discussed in Chapter 2. However, given the relationship between the number of vegetative tillers and the number of flowering tillers, along with the vernalisation mechanism determining flowering tillers, it is probable that a reduction in tiller number and growth rate would also have been found in the studies on the *parent clonal library* plants.

5.6.3 (ii) Are evolutionary responses adaptive under simulated drought?

For evolution in response to environmental change to be considered adaptive, there must be a home-environment fitness advantage, i.e. plants of drought ancestry should do better under drought than plants of control ancestry (Hendry *et al.* 2010). Although our results support evolutionary change in *F. ovina*, they provide little support that this change is adaptive, at least as defined in this way. If we had detected a classical signature of local adaptation, we would have expected to observe plants of drought ancestry either growing significantly more tillers under drought, or re-growing their biomass more quickly following the drought, compared to plants of control ancestry. We did not observe these

patterns. The evolution of a slower tiller growth rate may imply that plants of drought ancestry are producing tillers at a rate that can better be maintained under drought conditions. However, in the timescale of our experiment, this did not lead to an increase in tiller number for plants of drought ancestry under the drought treatment.

A comparison of the change in percentage living canopy surface (Figure 5.8 **A**) with the change in tiller number (Figure 5.8 **B**), suggests that plants from the drought and control climate ancestries differ in how they manage senescence of leaf tissue at the tiller level during a drought. Figure 5.8 **A** shows that there is a significantly reduced rate of increase in percentage living canopy surface in plants of drought ancestry under drought conditions relative to control conditions. However, Figure 5.8 **B** shows that plants of drought ancestry did not suffer a reduction in tiller growth under drought conditions relative to control conditions. Percentage living canopy surface and change in tiller number reflect contrasting aspects of growth during the drought treatment. The former describes changes in quantity or proportion of living plant tissue. The latter describes changes in the modular architecture of the plant. Plants of control ancestry appear to respond to drought by restricting the initiation of new tiller growth, whereas drought ancestry plants appear to have a lower growth rate under control conditions, but are able to maintain tiller growth under drought (Figure 5.8 **B**). The suggestion that plants from drought and control ancestries differ in the way they manage leaf senescence through a drought, is supported by the change in living canopy per tiller from the start to the end of the drought (Figure 5.9 **B**). A value closer to zero indicates that the rate at which the proportion of living tissue changed through drought is equal

to the rate at which tillers changed. The average change in the percentage of living canopy per tiller is closer to zero for plants of drought ancestry, compared to those of control ancestry under drought, however this difference is not statistically supported (Figure 5.9 **B**). In summary, these results suggest that under drought conditions, plants of drought ancestry are still senescing a high proportion of their leaves, but may derive associated benefits through tiller survival. Senescence can in itself be an adaptive strategy to survive drought (Munné-Bosch & Alegre 2004; Volaire & Norton 2006). Volarie *et al.,* (1998) found that recovery following drought was dependent on the ability to maintain tillers through a drought in *Dactylis glomerata* and *Lolium perenne*. Under the level of water deficit imposed by our drought treatment, *F. ovina* plants were easily able to recover; all plants showed some regrowth of above-ground biomass a month after the drought treatment. Compensatory growth, such as that observed in the greater regrowth biomass from plants that had been under simulated drought, has been observed in studies of other grasses (Volaire, Thomas & Lelièvre 1998). This may be as a result of the "Birch effect" which is the nutrient pulse generated by soil carbon and nitrogen mineralisation following the rewetting of soils (Jarvis *et al.* 2007; Lado-Monserrat *et al.* 2014).

On average all the plants grew through the drought, whether measured by the change in the number of tillers, or the percentage living canopy surface. The data from the percentage living canopy surface measurements suggest that drought-treated plants started growing in the second half of the experiment, after the 1st August. This flush of growth in the second half of the treatment is likely to have resulted from the methods used to control the level of moisture deficit in the

drought treatment. This was an unavoidable consequence of using a microcosm-type experiment compared with a drought treatment applied to an intact grassland (as at BCCIL).

We observed a trend for a reduction in the number of flowers produced in plants of drought ancestry compared to plants of control ancestry. We know from the results of Chapter 2 that the number of flowers is strongly correlated with male reproductive success. Therefore a reduction in flower number is also likely to reduce male reproductive success. We have now observed reduced fitness in two different components of plant fitness: growth rate and male reproductive success. Results from our microcosm experiment did not support a significant difference in the number of seeds produced by plants of drought ancestry compared to plants of control ancestry. The data for the average number of seeds per flower shows no clear pattern. Examining the number of flowers produced in 2016 (the flowering season that followed the simulated drought treatment), we found no evidence that plant fitness through sexual reproduction was affected by the drought treatment, or that plants of different ancestries differed in the number of flowering tillers they produced. The inconsistency between the patterns of reproductive success that we observe from one year to the next highlights the complexity of determining lifetime fitness in long-lived plants with modular growth forms. We do not know how a reduction in a fitness component in one year may be balanced by fitness gains in another year, or the relative importance of different fitness components (such as asexual reproduction, male reproductive success, female reproductive success, survival) for a plant's total lifetime fitness. There are also important components of plant growth and fitness that we have not measured here. We have not integrated

early-acting life-history traits or male reproductive success within this experiment. Also, we have only measured above-ground biomass, and there could be evolutionary responses in root biomass or root architecture that we did not observe. These plants are long-lived, and so total lifetime fitness will be the accumulation of offspring and survival over many years of growth and reproduction.

To our knowledge, two other studies have examined adaptive responses to climate change in the genus *Festuca*. These studies examined the local adaptation of populations to environmental stresses likely to be altered by climate change in *Festuca eskia* and *Festuca lenensis*. These species are both dominant tussock forming species, fulfilling equivalent ecological roles to *F. ovina* in their native habitats and have with similar growth forms and reproductive biology (Gonzalo-Turpin & Hazard 2009; Rilke & Najmi 2011; Liancourt *et al.* 2013). Gonzalo-Turpin and Hazard (2009) used a reciprocal transplantation approach to study local adaptation of the alpine fescue, *Festuca eskia*, along an altitudinal gradient. High altitudes are generally harsher environments than low altitude sites, evidenced by smaller plants with lower reproductive investment. At high altitudes, 'home' plants had higher survival than 'away' plants, but there was no difference between 'home' and 'away' plants in survivorship in low altitudes (less harsh environments) dependent on the plant's origin. At low altitudes, reproductive output was highest in plants of the 'home' habitat, but high altitude plants did not have higher reproductive output in their 'home' altitude. The authors concluded that different components of plant fitness were responsible for adaptation under different levels of stress, either through survival or reproductive output. In a similar study focusing

on *Festuca lenensis,* Liancourt *et al.* (2013) investigated local adaptation along an aridity gradient varying with altitude, in a Mongolian steppe habitat. They also found evidence of local adaptation, expressed through different components of fitness under different levels of stress. On the wetter, lower slope, 'home' plants had greater above-ground biomass whereas, on the upper, drier slope, 'home' plants showed greater survival. These studies both suggest that under more stressful conditions survival is the key fitness component, whereas under less stressful conditions reproductive output or growth rate are more important. The drought treatment imposed during this experiment was not sufficiently severe to cause mortality in *F. ovina*. Applying a more severe drought could clarify whether traits evolved under a historical drought treatment in *F. ovina* are adaptive under drought by increasing survival.

5.6.4 (iii) What are the consequences of evolutionary change for co-existing species?

We have documented a strong negative correlation between the vegetative biomass produced by *F. ovina* and the vegetative biomass produced by *K. macrantha*. This suggests that there is strong competition between *F. ovina* and *K. macrantha*, likely to be due to the denial of resources potentially available to one of the competing individuals by the other. Our results have also shown that under control conditions *F. ovina* of pure drought ancestry has evolved to have a reduced vegetative biomass. This suggests that climate-induced evolutionary change in biomass production in *F. ovina* is likely to provide a competitive opportunity for *K.*

macrantha, demonstrating how evolutionary responses in one species, may alter competitive interactions with other co-existing species.

Simulation studies that investigate the eco-evolutionary responses of species to climate change are beginning to incorporate co-existing species into their models (de Mazancourt, Johnson & Barraclough 2008; Norberg *et al.* 2012). However, these studies make the assumption that when populations are adapted to climate change this will result in a higher growth rate and improved competitive fitness, leading to competitive exclusion of species or genotypes poorly adapted to the climate (de Mazancourt, Johnson & Barraclough 2008; Norberg *et al.* 2012). However, our results suggest that there is a trade-off between climate adaptation and competitive ability, and that evolution under climate change can result in a reduced growth rate, which will reduce a plant's competitive ability. Therefore, at least in some species, the assumption that evolutionary responses to climate change will improve competitive ability is unjustified.

There was no evidence of a correlation between the vegetative biomass of either *C. panicea* or *C. caryophyllea* with the vegetative biomass produced by *F. ovina*. One possible explanation is that the different growth patterns of the grasses and sedges enabled the sedge species to better avoid direct competition with *F. ovina*. In the *Carex* species used in this experiment new ramets do not grow directly next to their parent ramet. In the grass species used in this experiment, new tillers grow directly next to their parent tiller, forming close dense tussocks. After nearly two years of growth, the microcosms still had visible bare ground, and so it may be that the *Carex* species were able to avoid more direct competition (which we would have expected to have an affect on vegetative biomass) by growing in areas further

away from the grasses. The two grass species were not able to do this, and so may have been in more direct competition with each other – in many cases tussocks of the two grass species became interdigitated. In natural calcareous grasslands co-existing species form a dense, repeating matrix, with a closed canopy (Booth & Grime 2003). Although different growth patterns might reduce direct competition between co-existing species, the possibility of avoiding other species is much more limited in natural grassland communities than in our microcosms.

Individual plant clonal lines can exhibit specific responses to competition (Fridley, Grime & Bilton 2007). The clonal line of *C. caryophyllea* used in our study (Cc09) has been shown to interact strongly in competition with clonal lines of *K. macrantha* (Fridley, Grime & Bilton 2007). In our experiment, the vegetative biomass for this single clonal line of *C. caryophyllea* (Cc09) ranged from 0 – 103.9 mg. This huge variation in vegetative biomass suggests that genotype specific responses to the other species in the microcosm are occurring, or that there are residual establishment effects. Twelve different clonal lines of *K. macrantha* and 18 different clonal lines of *C. panicea* were used in our study and were allocated to microcosms, randomised within each blocking replicate and treatment group. This set-up means that there is not enough replication in our microcosm study to test whether the outcome of competition depends on (clonal) genotype × genotype (g × g) interactions. However, given the results of studies such as Fridley, Grime & Bilton (2007) which demonstrate the strength of genotype specific interactions between co-existing grassland species, we hypothesise it is likely that such g × g interactions will influence the responses of grassland communities to climate change.

5.6.5 Conclusions

We have documented the evolution of *F. ovina* in response to drought at BCCIL through a reduction in tiller growth rate and vegetative biomass. This is accompanied by a trend for reduced reproductive output. However, there is little evidence that these evolutionary responses are adaptive under a short-term drought treatment. We have also identified a strong negative correlation between the vegetative biomass of *K. macrantha* and *F. ovina*. Given the evolution of a reduction in biomass in *F. ovina,* this suggests that competitive interactions between co-existing species may be altered in response to climate change. These results indicate that evolutionary responses to climate change may alter species interactions in unanticipated ways.

5.7 References

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6 Chapter 6. General discussion and conclusions

6.1 Abstract

Anthropogenic climate change is altering temperature and weather patterns across the globe. Adaptive evolutionary responses may provide one mechanism by which plants can persist through rapid climate change. In this thesis we have used a long-term climate change experiment to examine evolutionary responses to climatic selection in *Festuca ovina.* We have assessed the sources of genetic variation for evolutionary responses in *F. ovina* (Chapters 3 and 4). We have demonstrated that a number of traits, expressed across the life-stages of *F. ovina*, have evolved in response to climate change (Chapters 2 and 5). However, we have not found evidence that the evolutionary responses that we have observed are adaptive when under an experimentally simulated drought (Chapter 5). We have shown that climatic selection has driven reductions in two key components of fitness, male reproductive success and tiller growth rate, reducing potential reproductive rate and growth. Finally, we have shown how evolutionary responses in one species may alter interactions with other, co-existing species (Chapter 5). Here we synthesise the results of this thesis, examining the different processes influencing evolutionary responses to climate change in the BCCIL population and consider how these responses affect the community as a whole.

6.2 Sources of genetic variation in *F. ovina*

6.2.1 Heritable genetic variation

Standing quantitative genetic variation represents a fundamentally important component of the capacity for populations to adapt to environmental change, and has been studied extensively. In Chapter 3 we showed that there is significant heritable genetic variation in a wide range of morphological and reproductive traits in *F. ovina*. The heritability of some traits is low, particularly reproductive traits. The presence of heritable genetic variation provides the raw material for evolutionary responses. However, as we go on to discuss, many other processes will determine whether evolutionary responses will occur in response to climate change and, if they do, whether they are adaptive.

6.2.2 Maternal effects

The quantitative genetic analyses conducted in Chapter 3 revealed that a large proportion of phenotypic variation in *F. ovina* is the result of *maternal effects*. Maternal effects may provide a particularly effective source of adaptive phenotypic variation for plant populations that are characterised by high levels of gene-flow among populations that have limited seed dispersal capacity, and which occupy highly heterogeneous habitats (Galloway & Etterson 2007). Under such conditions, gene-flow from other parts of the habitat may be maladaptive and the maternal environment will represent a more similar environment to that in which the seedling must establish, in comparison to the paternal environment. Therefore maternal effects that improve the offspring's fitness for growth under the same

conditions as the mother will be adaptive (Mousseau & Fox 1998; Galloway & Etterson 2007). These are the conditions found at BCCIL for *F. ovina*. There is high gene-flow among individuals in the different experimental treatments, combined with a highly heterogeneous habitat, and seed dispersal is limited. This means that the conditions experienced by an individual in one part of the habitat may be very different to those experienced in another part of the habitat. Thus, maternal effects may provide an important, though understudied, mechanism for plant adaptation to climate change, both at BCCIL, and more widely (Walter *et al.* 2016).

6.2.3 Intraspecific variation in genome size

The final source of genetic variation that we have identified in *F. ovina* is intraspecific variation in genome size (Chapter 4). This is most likely the result of the replication and transposition of repetitive elements, particularly retrotransposons, which have a high frequency in grasses (Gaut 2002). Retrotransposons and transposable elements can influence adaptation through several mechanisms. They can facilitate the horizontal transfer of new genes, they can induce mutations which provide adaptation to particular environments, and they can alter gene regulation in response to particular environmental stressors (Casacuberta & González 2013). Although we do not know the specific cause of intraspecific variation in genome size in *F. ovina*, it may represent a novel source of variation for evolutionary responses.

6.2.4 Sources of genetic variation and evolutionary responses

In this study we have identified three key components of genetic variation that may provide a source of variation for evolutionary responses to climate change in *F. ovina*; heritable genetic variation, maternal effects and intraspecific variation in genome size. Although discussed separately, these sources of variation are not independent of each other, and all may act to drive adaptation. Maternal effects, may be particularly important in providing an initial flexible mechanism for adaptation (Galloway & Etterson 2007). The traditional view of evolutionary responses to climate change, focusing solely on standing heritable genetic variation, may miss other important components of genetic variation that can contribute to adaptive responses to climate change in natural populations.

6.3 Evolutionary responses to climatic selection

6.3.1 The evolution of traits in F. ovina

Through the experiments conducted in this thesis, we have documented evolutionary changes in response to 17 years of simulated drought treatment in a suite of traits in *F. ovina*. In Chapter 1, we set out the criteria for demonstrating evolutionary responses to climatic selection; namely, that (i) there is heritable genetic variation in the trait of interest, (ii) the trait is under climatic selection, and (iii) there is a difference in the value of the trait as a result of changes in climate.

Our results in Chapter 2 strongly suggest evolutionary changes in male reproductive success and germination latency. However, these traits were

measured on plants collected from the field, and on seed germination traits, which can be considered to be phenotypes of the parent plant, and not the offspring (Galloway, Etterson & McGlothlin 2009). Consequently, the responses we observed may still be the result of carryover effects from the field, despite our best efforts to minimise these effects (plants grown in a common garden environment for 3 years prior to the collection of seed).

The phenotypic changes documented in Chapter 5 fulfil the criteria for demonstrating evolutionary responses to climate change. We have shown, in F1 progeny, that there is trait differentiation between plants subjected to drought and control treatments, and that these traits are heritable. These evolutionary changes have resulted in a slower tiller growth rate and reduced biomass in plants of drought ancestry at BCCIL, compared to those with control ancestry. These results demonstrate the potential for rapid (17-year) evolution to climate change in a perennial grass species such as *F. ovina*, and indicate that evolutionary change may provide a mechanism for the persistence of plant populations through climate change.

A limitation of the experimental drought treatment is that, although our intention was to alter only one environmental variable, water availability, by using shelters to impose the drought, multiple other environmental conditions (including temperature, humidity and light) were also altered. Collectively this combination of altered environmental conditions may have changed plant growth and functioning in response to the drought treatment in ways different to those if only water availability had been altered. This limits the extent to which these results can form a basis for predictions about natural grassland communities.

6.3.2 Life-history strategies and adaptation

Adaptive evolution is defined as evolution that results in a fitness benefit in the new environment (Franks, Weber & Aitken 2014). Evolutionary responses to climatic selection are not necessarily adaptive, and may result instead from genetic drift (Merilä & Hendry 2014). We tested the adaptive nature of evolutionary responses through the application of a drought treatment to F1 *F. ovina* plants with ancestry in drought-treated and control plots at BCCIL (Chapter 5). We found little evidence that the evolutionary changes we have observed were adaptive under the simulated drought treatment. We did not see an increase in reproductive, survival-related or size-related traits in plants with drought ancestry, as would be expected if these traits were adaptive under drought. However, we did find that tiller growth, while lower in plants with drought ancestry than those from control plots, is maintained under drought treatment in these plants. In contrast, plants with ancestry in the control plots at BCCIL suffered a significant reduction in tiller growth rate under a simulated drought. It is unclear how these evolutionary changes will affect plant fitness over the full lifespan of *F. ovina*.

Plant fitness in perennial species involves a number of distinct components, that relate to survival (germination success, seedling establishment, asexual growth) and current and future reproductive success (female and male reproductive output, asexual growth). The ultimate measure of plant fitness is the total number of progeny descended from a plant over its complete lifespan. The relative importance of the underlying fitness component traits will vary depending on the level of environmental stress and the strength of biotic interactions such as competition. It may be that a fitness component that we did not measure, such as

survival, is most important under drought. Thus, the evolutionary responses that we have documented may indeed be adaptive if fitness could have been measured over the entire lifetime of experimental plants, and if we had monitored all the traits which contribute to fitness.

In response to a long-term drought treatment we expect to see trait differentiation between plants from the drought and control treatments. Under drought conditions, we expect selection to shift phenotypes towards life-history strategies that are adaptive under drought, specifically traits associated with either *drought tolerance*[6] or *drought escape* strategies. Within the *C-S-R* life-history strategy framework proposed by Grime (1974), drought tolerance traits are consistent with a stress-tolerant strategy, and drought escape traits are consistent with a ruderal strategy. Therefore, we can ask to what extent the trait differentiation that we observed fits with these strategies.

We found that tiller number was negatively genetically correlated with seed production (Chapter 3). We have also found that evolutionary change driven by drought conditions at BCCIL has reduced tiller growth rates and tiller number. These results suggest that as selection reduces tiller number, we may see a correlated increase in seed output, which, in turn, would be consistent with evolution towards a more ruderal, drought escape strategy, where high seed output is a priority. However, we found no consistent pattern of differentiation in seed production between plants of drought or control ancestry in the experiments carried out for this thesis. Also consistent with traits shifting towards a ruderal

[6] Words in italics are defined in the glossary.

strategy is evidence from the *parent microcosm experiment*, which found that flowering time is earlier in plants from drought plots at BCCIL (R. Whitlock, personal communication). Earlier flowering allows plants to complete flower production and to set seed before the onset of the drought. In another study on the BCCIL study system, Ravenscroft, Fridley & Grime (2014) found that *Plantago lanceolata* had shifted traits in a direction consistent with a drought avoidance strategy under drought-treated conditions. The study by Ravenscroft et al. (2014) was carried out on clonal lines of plants collected from the field, therefore, although these results are indicative of evolutionary responses, they may still be carryover effects from the field. We now have two examples from the BCCIL study system (in two separate species) that suggest evolution towards a ruderal, drought escape strategy.

6.3.3 Evolutionary processes

A number of different processes contribute to the evolutionary responses that we have observed at BCCIL. Assortative mating, for example, is significant because it can lead to reproductive isolation (Fox 2003; Weis *et al.* 2005). We have shown that mating in *F. ovina* is assortative with respect to flowering time (Chapter 2). We have found that, in *F. ovina* from the BCCIL system, plants from the drought treatment flower significantly earlier than plants from the control treatment (R. Whitlock, unpublished data). Therefore, given that plants mate assortatively by flowering time, we might expect over time to see partial reproductive isolation between plants from drought and control treatments at BCCIL. This could reinforce

evolutionary responses and the phenotypic differentiation of other traits under climatic selection.

Another important process influencing evolution is the underlying genetic architecture among traits (Chapter 3). Genetic correlations that are antagonistic to the direction of selection will constrain the evolution of those traits (Etterson & Shaw 2001). We have identified negative genetic correlations between several pairs of traits. Of particular interest is a negative genetic correlation between tiller number and number of seeds, which indicates a fundamental constraint between asexual and sexual reproduction. This defines a classic genetically based trade-off between these aspects of fitness and means that the evolution of plants that reproduce both sexually and asexually may be constrained along the annual-perennial spectrum.

6.4 The responses of populations and communities to climate change

6.4.1 Population persistence under climate change

Climate-induced reduction of individual fitness may lead to negative population growth rates, and eventually to the local extinction of populations (Anderson 2016). We have shown that fitness has decreased in two key fitness components in *F. ovina*: male reproductive success and tiller growth rate. If there is no compensatory increase in other fitness components, then plant total fitness may be reduced, allowing for possible population-level decline. We have found no

evidence for evolutionary changes in flower number and seed number that would compensate for these fitness losses. However, other components of fitness that we have not measured, such as survival or seedling establishment, might have evolved to compensate. One of the difficulties of studying long-lived perennial plants is that plant total fitness is the accumulation of many years of growth and reproduction. In our study we have only been able to study any trait for, at most, 3 years (flower and seed number; Chapter 3). Therefore we do not know how, in the long-term, plant total fitness will be affected by the declines that we have observed here.

6.4.2 Integrated ecological and evolutionary processes

The responses of plant communities to climate change will depend upon both ecological and evolutionary processes (Lavergne *et al.* 2010). In Chapter 5 we conducted an experiment using a microcosm community. This experiment investigated whether phenotypes altered by climate-driven evolution are expressed in the presence of a community of other species, and whether evolutionary responses in one species alter interactions with others. Our results show that evolutionary differentiation in traits can be observed in a community context, despite the fact that no such differentiation could be observed when plants were grown in isolation (Chapter 3). Other studies have also shown that the presence of co-existing species can be important for the detection of evolutionary responses, and detecting the magnitude of that response (Bischoff *et al.* 2006; Ariza & Tielbörger 2011; Tomiolo, van der Putten & Tielbörger 2015). We cannot specifically say that it was the presence of co-existing species that resulted in the detection of

evolutionary responses because abiotic growth conditions also differed between the experiments carried out in Chapters 3 and 5. However, it is likely that the presence of co-existing species was a factor in the detection of these evolutionary responses. Moreover, our results show that climate induced evolutionary changes are strong enough to be expressed in the modified competitive environment provided by neighbouring plants. Given that genetic variation is important for the outcome of competitive interactions in species rich grasslands (Fridley, Grime & Bilton 2007), this makes the evolutionary responses we have observed of potential ecological importance.

As phenotypes change in response to climatic selection, this can alter species interactions with other co-existing species. In Chapter 5, we showed that competitive interactions between *F. ovina* and *Koeleria macrantha* may be altered by evolutionary change in *F. ovina* (Chapter 5). The biomass of *F. ovina* is strongly negatively correlated with that of *K. macrantha*, suggesting strong direct competition between the two species. As biomass has evolved to be lower under drought conditions in *F. ovina*, this may provide a competitive opportunity for *K. macrantha*. This result suggests that there is a trade-off between climate adaptation and competitive ability, demonstrating the necessity for research that integrates trade-offs and feed-backs between ecological and evolutionary processes. Such studies are crucial if we are to understand and predict the responses of populations and communities to climate change.

6.4.3 Responses of communities to climate change

In the introduction to this thesis, we discussed the range of responses that have been observed in plant communities subjected to experimental climate change. Some communities have shown little change (Grime *et al.* 2008; Tielbörger *et al.* 2014), while for others, climate treatments have resulted in reduced species richness and altered community composition and ecosystem function (Peñuelas *et al.* 2007; Evans *et al.* 2011; Fay *et al.* 2011). The BCCIL study system has seen relatively little change, despite the application of strong experimental climate change treatments. However, there has been significant reorganisation of species abundances at fine-scales within the BCCIL plots (Fridley *et al.* 2011). *Festuca ovina* has increased in abundance in the drought plots. This appears to contradict the results we have found here, which have shown declining fitness in key traits, including tiller growth rate and vegetative biomass, indicating evolutionary responses that are potentially maladaptive. However, it should be noted that although tiller growth rate decreased under drought, it nonetheless remained positive during the drought treatment. The ability to maintain growth through drought conditions may provide *F. ovina* with an advantage if the decline in its average growth rate is not as strong as that of other species during the drought. If this were the case, then this challenges our concept of an adaptive response, the classical definition of which is a fitness benefit in the new environment. Instead, an adaptive response within an ecological community may be fitness that is greater relative to the fitness of other co-existing species. Alternatively, the abundance of *F. ovina* within the drought plots at BCCIL could increase if regeneration from seed is greater under the drought conditions in comparison to control conditions. The

decline in abundance of other species within the drought plots may provide additional bare ground and more opportunities for regeneration from seed for *F. ovina* (Fridley *et al.* 2011). These two options highlight the importance of studying evolutionary responses to climate change within the context of the community of co-existing species in order to understand fully the responses that we observe at the level of the whole plant community.

6.5 Future directions

This study has added significantly to our understanding of the evolutionary responses to climate change occurring within a unique experimental climate change manipulation applied to an intact ecosystem at BCCIL. However, it also leaves many areas for further study. One area that is a priority is what constitutes 'fitness' in a long-lived clonal perennial plant, and what fitness components are of most importance for plant total fitness under different circumstances. This would require monitoring growth and reproductive output in *F. ovina* for many years, or using modelling techniques, such as those used in aster models (Geyer, Wagenius & Shaw 2007), to model the life-history of *F. ovina*. It is also important that we consider how fitness in *F. ovina* is changing, relative to the fitness of other species within the community. This would require monitoring the rates of reproductive and vegetative output of the other species in the BCCIL community over multiple years. Further work is still required to determine whether, over the lifespan of *F. ovina*, the

evolutionary responses that we have observed have increased plant total fitness under drought conditions.

Another interesting area for further study would be to assess whether the evolutionary responses that we have observed in early-acting life-history traits are adaptive in the field. We have found that time-to-germination has evolved to be later in plants of drought ancestry (Chapter 2). However, these measurements were taken under lab conditions. In the field, numerous other factors will influence germination. We do not know whether later germination will be adaptive under field conditions and, if so, how this will alter patterns of regeneration from seed in *F. ovina*. One way to carry this out could be to plant seed into established microcosm experiments (such as those established in Chapter 5), and monitor the germination and establishment of the seed under control and drought conditions. Furthermore, it would be interesting to understand how maternal effects may provide adaptive phenotypic variation. Maternal effects are likely to be strong in early-acting life-history traits (reviewed by Roach & Wulff 1987), and may be particularly important for initiating seed germination in heterogeneous environments. We also do not yet know to what extent the maternal effects we have documented are genetically or environmentally determined.

There is much scope for further work on the integrated roles of ecological and evolutionary processes in determining the responses of populations and communities to climate change. Here we have demonstrated that evolution may alter competitive interactions between species; however, we have only looked at three pairs of plant-plant interactions. There are numerous other biotic interactions, such as plant-insect, plant-mycorrhiza, plant-microbe, as well as many

other plant-plant interactions, which may alter, or be altered by, evolutionary responses to climate change. The feedback between evolutionary and ecological processes has the potential to alter significantly the responses to climate change that are predicted where only a single species has been investigated. Therefore, it is vital that more research is carried out in this area.

Here, we have focused on a single climatic factor: drought. However, climate change will alter many combinations of climatic variables, including the intensity and frequency of precipitation events, temperature, and atmospheric CO_2 concentration. In a meta-analysis of experimental climate manipulation studies Wu *et al.* (2011) found that biotic responses to multiple climate change factors were not predicted as an additive sum of single factor responses. Therefore, more studies of multifactorial treatments will be essential to predict reliably the responses of plant populations and communities to climate change. It is also important to understand how the year-to-year variability of changing climate patterns will alter responses, and particularly how this may disrupt evolutionary responses (Etterson 2004; Thompson *et al.* 2013).

6.6 Conclusion

In this thesis, we have used a unique long-term experimental climate change study to investigate evolutionary responses to climate change in *F. ovina*. We have shown that there is heritable genetic variation in traits important for adaptive responses to climate change and have demonstrated that traits, including

germination time and biomass production, across the lifecycle of *F. ovina* have evolved in response to climate change. However, we have limited evidence that evolved phenotypes are adaptive under the environmental conditions imposed in our experiments. We have observed decreased fitness in two key components of total plant fitness, which may have consequences for the persistence of *F. ovina* populations under climate change. We have also shown that evolutionary responses to climate change may alter interactions between species, specifically competitive interactions. This result challenges the assumption that adaptation to climate change will result in a competitive advantage, and may undermine the ability of populations to withstand competition. Our results demonstrate that evolutionary responses can provide populations with a mechanism to persist through climate change, but that evolutionary responses may have wider ecological impacts, including effects on biotic interactions. Therefore, integrated studies, incorporating both ecological and evolutionary processes, are essential in order to better understand and predict the responses of plant populations and communities to climate change.

6.7 References

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A1 Appendix 1 – Glossary

A1.1 General terms

C-S-R triangle The life-history strategy ordination system proposed by Grime (1974) which locates plant phenotypes into competitor, stress-tolerant and ruderal strategies.

Direct genetic effects The influence of an individual's genes on its own phenotype (Whitlock *et al.* 2011).

Drought escape The ability of a plant to set seed and complete its lifecycle before the onset of drought (Farooq *et al.* 2012).

Drought tolerance The ability of a plant to survive under low water availability through morphological and physiological adaptation by regulation of the effects of water (Farooq *et al.* 2012).

Inter-specific indirect genetic effects The influence of an individual's genes on another individual's phenotype (Whitham *et al.* 2006).

Local adaptation The relative fitness benefit of a resident population in their local environment when compared with a non-resident population (Kawecki & Ebert 2004; Leimu & Fischer 2008).

Maternal effects The effects of maternal traits on an offspring's phenotype that is not the result of the direct inheritance of genes from the mother (Mousseau & Fox 1998; McAdam, Garant & Wilson 2014).

Maternal environmental effects Maternal effects that are the results of the environment causing variation among mothers (Kruuk & Hadfield 2007; McAdam, Garant & Wilson 2014).

Maternal genetic effects Maternal effects that are the result of a mothers genotype (Kruuk & Hadfield 2007; McAdam, Garant & Wilson 2014).

Phenotypic plasticity The production of different phenotypes under different environmental conditions, from a given genotype (Merilä & Hendry 2014).

Resistance The extent to which a community remains unchanged despite perturbation (Hughes & Stachowicz 2004; Reusch *et al.* 2005).

Resilience The rate of recovery of a community to its state preceding the perturbation (Hughes & Stachowicz 2004; Reusch *et al.* 2005).

Thermophilisation An increase in the dominance of warm-adapted species within communities (De Frenne *et al.* 2013).

A1.2 Study system specific terms

Ancestral climatic environment The climate treatment received by an individual plants parents at BCCIL (either drought, hybrid or control).

Simulated drought treatment The drought treatment applied to the microcosm experiment discussed in Chapter 5, which was carried out at Ness Botanic Garden during the summer of 2015 (either drought or control).

Hybrid ancestry Offspring for whom one parent originated from a control plot, and one parent originated from a drought plot at BCCIL.

Offspring clonal library The F1 progeny array generated from the parent clonal library.

Parent clonal library A collection of 59 clonal lines of *Festuca ovina* collected from the drought and control treatment plots at BCCIL and now maintained as clonal lines at Ness Botanic Gardens.

Parent microcosm experiment An experiment in which clonal replicates of *F. ovina* from the parent clonal library were grown in a common environment and their phenotypes measured.

Pure control ancestry Offspring for whom both the maternal and paternal parent originated from control plots at BCCIL.

Pure drought ancestry Offspring for whom both the maternal and paternal parent originated from drought plots at BCCIL.

A2 Appendix 2

A2.1 Collection and propagation of *F. ovina* clonal lines

In July 2010, *F. ovina* individuals were collected from drought and control plots at BCCIL by R. Whitlock, after 17 years of climate manipulation. Thirty individuals were collected from each of these climate environments (drought and control; six individuals per plot, per treatment). A stratified random sampling design was used to collect the plants. Each plot was 3 m × 3 m. The 2 m × 2 m region at the centre of each plot was split into sixteen 0.5 m × 0.5 m quadrat areas, one was selected at random and this was divided into twenty-five sub-quadrats of equal area. One of these 0.1 m × 0.1 m sub-quadrats was selected at random and the *F. ovina* individual closest to the centre was selected. If an individual of *F. ovina* could not be found in the first sub-quadrat, then another sub-quadrat was selected randomly from the same quadrat, until a single individual had been collected from the quadrat. This process was repeated until six *F. ovina* individuals had been sampled from the plot. Once an individual had been selected it was removed from the soil and potted into a cell tray. The soil depth at each individual's position was recorded twice, and an average taken. Soil depth varies considerably within plots (from bare rock to ~40cm), and is an important factor driving fine-scale plant community structure (Fridley *et al.* 2011). Each plant's spatial location was also recorded (to the nearest centimetre), relative to the northwest corner of the plot from which it was sampled.

A2.2 Genomic DNA extraction methods

DNA was extracted from 2–5 dried leaves (approximately 4–10 mg) using a high-throughput micro-titre plate-based protocol modified from Whitlock *et al.* (2008). Dried plant material was ground using a single 5 mm stainless steel bead (Qiagen, UK) in a 2 ml round bottomed tube (Eppendorf, UK) in a mixer-mill (Tissue Lyzer II, Qiagen, UK) at 25hz for 5 minutes. The ground plant material was incubated in 225 µl of extraction buffer (SEB01; 100 mM tris (pH 7.4), 500mM NaCl, 50 mM EDTA, 0.7% SDS, 52 mM Na_2SO_3 (Baranwal, Majumder & Singh 2003)) containing 4 µl ProK and 1 µl RNAseA (Qiagen, UK) for 30 minutes at 55 °C. Precipitation buffer, 225 µl (3.6M Potassium; 6M acetate), was added and the tube was centrifuged at 13000 rpm for 15 minutes. The supernatant was transferred to a 1.2 ml low profile storage plate (ThermoFisher Scientific, UK) and 1.5 × volume of binding buffer (6 M NaI (Elphinstone *et al.* 2003)) added and mixed well by pipetting. This mixture was loaded onto a Unifilter Whatman filter plate (GE Health Life Sciences) mounted on top of a 1.2 ml storage plate that was centrifuged in a plate centrifuge at a Relative Centrifugal Force (RCF) of 40 *g* for 3 minutes (this process was repeated to filter all the sample mixture through, with the waste filtrate being discarded after each centrifuge). 750 µl of wash solution (10 mM Tris, 0.5 MM EDTA, 50 mM NaCl, 50% ethanol (Elphinstone *et al.* 2003)) was added to the wells of the filter plate and centrifuged at an RCF of 159 *g* for 5 minutes. This was repeated. Finally the plate was centrifuged at an RCF 2137 *g* for 2 minutes and then left to dry at room temperature for 3 hours. DNA was eluted in 100 µl low TE buffer warmed to 80°C (10mM tris-HCl pH7.4, 0.1 mM EDTA).

A2.3 Primer screening and PCR methods

Microsatellite fragments were separated by length using capillary gel electrophoresis on an ABI Prism 3130 XL. Microsatellite allele fragment lengths were determined using an internal size standard (LIZ 500; ThermoFisher Scientific, UK). PCR products were diluted 1:4 in low TE buffer and 0.6 µl of each PCR reaction was mixed with 9.32 µl of formamide (ThermoFisher Scientific, UK) and 0.08 µl of GS500 LIZ size standard (ThermoFisher Scientific, UK) in a 96 well, semi-skirted PCR plate (StarLab, UK; 10 µl volume, total). The plate was covered with a silicon sealing mat (Corning Incorporated, USA) and PCR products were denatured by heating to 95°C for 3 min 20 sec, before quenching for 2 minutes on ice. Fragments were sized with reference to the internal LIZ size standard, using GeneMapper Version 3 software (ThermoFisher Scientific, UK).

Twenty-three of the primer pairs amplified fragments of the expected size. Of these, 14 were either monomorphic, showed little variation or had inconsistent amplification. Primer pairs were excluded if they failed to amplify in less than half of a set of eight test individuals from the parent clonal library (inconsistent amplification), or if allele peaks could not be reliably identified over repeated runs of the same set of samples (poor amplification). This left 9 primer pairs that amplified consistently and that showed high levels of polymorphism within a batch of test individuals.

A2.4 Microsatellite scoring methods

Microsatellite loci were scored using semi-automated genotyping methods set up for each multiplex within GeneMapper Version 3 (Applied Biosystems). We made up to four allele calls for each locus per individual, since our population of *F. ovina* is tetraploid (see Chapter 4). An analysis method was created for scoring the microsatellite data from each multiplex using a test batch of 40 samples. Each analysis method used the "default method" as a template from which to create the analysis method. The maximum expected number of alleles was set to 4. The analysis method was then linked to a panel containing allele bins for that multiplex. Bins were added automatically using the "auto-bin" setting and 40 samples as reference data. Bins were manually checked and, where necessary, additional bins were added or modified, if the auto-bin processes had missed any alleles.

Microsatellite scoring was conducted using the analysis method and bin set appropriate for each multiplex. The size standard was set to match the size standard we has used (LIZ 500; ThermoFisher Scientific, UK) and the data were analysed. Each size standard electropherogram was checked manually to ensure fragment sizes had been labelled correctly. In cases in which the size standard was highlighted as 'low quality' or 'check' by the sizing quality score in genemapper, the electropherogram of the size standard was checked, and where possible the sizing fragments were re-labelled correctly. If this was not possible then the sample was removed from the project and subsequently re-run.

A2.5 Parentage analysis error checking methods

A final set of error checking was carried out on the genetic dataset, to test the accuracy of allele calls. This used a series of random and directed error checks, and also a preliminary parentage analysis, to identify mis-called alleles. Fifteen individuals were selected at random from the dataset and the electropherograms for each of the 9 loci were re-examined, to check the accuracy of allele scoring. This found 2 instances in which an allele had not been called that should have been called, out of a possible 540 (15 indviduals, 9 markers, 4 possible allele calls per marker). Next a set of directed error checks were carried out based on the results from a preliminary parentage analysis. The preliminary parentage analysis was run with the following modifications: run for 1,300,000 iterations, with a thin interval of 1000 and a burn in of 300,000, with a sample size of 1000. A tuning parameter of E1=50 was specified to improve Metropolis-Hasting updates. From the pedigree provided by the preliminary parentage analysis 15 individuals were selected at random from those offspring that did not have a father assigned with > 80% confidence. For these 15 individuals the electropherograms for each of the 9 loci were re-examined, to check the accuracy of allele scoring. This also found 2 instances in which an allele had not been called but should have been out of 540 possible mistake options. Finally any allele that had been scored for less than three individuals was re-examined to check the validity of the microsatellite peak. This resulted in the removal of 5 alleles, and the re-assignment of 3 allele calls.

A2.6 Sensitivity analysis for parentage analysis

A sensitivity analysis was carried out to test the influence of the genotyping error rate estimation on the pedigree. The parentage analysis model was re-run with a fixed genotyping error rate. First the error rate was fixed to be effectively 0, with E1 = 1×10^{-10} and E2 = 1×10^{-10}. The pedigree estimated from this run had 358 out of 457 (78.3 %) exact matches in paternal assignment with the final pedigree. Of the 99 mismatches 26 were contributed by differences between the models in whether the model predicted a paternal parent, and 73 were direct differences in paternal assignment. Next the error rate was fixed at E1 = 0.1 and E2 = 0.005. The pedigree estimated from this run had 421 out of 457 (92.1 %) exact matches in paternal assignment with the final pedigree. Of the 36 mismatches, 21 were contributed by differences between the models in whether the model predicted a paternal parent, and 15 were contributed by direct differences in paternal assignment. Finally the sensitivity of the analysis to the Metropolis-Hasting acceptance rate was tested by re-running the model with a tuning parameter of 50. This pedigree had 430 out of 457 (94.1 %) exact matches in paternal assignment with the final pedigree. Of the 27 mismatches, 13 (2.8 %) were contributed by differences between the models in whether the model predicted a paternal parent, and 14 (3.1 %) were contributed by direct differences in paternal assignment.

A2.7 Flowering time data collection

To examine whether mating between plants from the *parent clonal library* was assortative with respect to flowering time we used data on the flowering

phenology of the *F. ovina* clonal lines collected from the *parent microcosm experiment*. In this experiment clonal replicates of the 60 plants in the parent clonal library were grown in a common environment at Ness Botanic Gardens, University of Liverpool, UK. Each clonal line had four replicates, each planted in a two litre pot in natural rendzina soil of 120 mm depth, on top of 60 mm depth of limestone chippings (total pot depth 180 mm). The clonal lines were planted in 2011 and flowering time data was collected in May-June 2013. Flowering was measured as first anthesis, which is defined as emergence of the first anther from any individual flower in the inflorescence. Flowering was monitored twice weekly. The minimum flowering time, the measure used in the assortative mating analysis, was calculated as the minimum flowering time within a pot, and then averaged across the replicates of that clonal line.

A3 Appendix 3

A3.1 Outlier removal from quantitative genetic analyses

Trait data for 4 of the parent plants and 15 of the offspring plants (that were initially planted in the heritability experiment) were not able to be included in the quantitative genetic analyses because either: when first planting there was not enough material to plant 4 tillers, they died during the course of the experiment, or during the course of the experiment they received excess nitrogen input from bird detritus. Bird detritus was deemed to have altered plant growth if tiller number increased by more than 20 tillers between consecutive tiller counts (which occurred approximately every three months).

A3.2 Estimation of leaf shape constant

In order to calculate the surface area of a *Festuca ovina* leaf we conducted a set of detailed measurements along the length of the leaf to calculate a "shape constant", to take into account the shape of the leaf along its length. We measured a sample of 20 leaves from the *parent clonal library* (11 and 9 plants originating from control and drought plots at BCCIL respectively). A measurement of leaf width was taken at the base, tip and 7 further points equidistant along the leaf. To calculate the shape constant we then scaled all of the leaf width measurements for each leaf relative to the width at the base of the leaf. This removed the size component of the overall shape in both the length and width dimensions to result in a "pure leaf shape". We then took the average of all of the scaled measurements

at each location along the length to find an average leaf profile. The area under this profile was calculated numerically by dividing the profile into parallelograms. The areas of these parallelograms were summed to give the scale-free area of an average leaf: this is the shape constant, calculated as 1.029. The shape constant, a, allows estimation of leaf surface area, SA, by multiplication with leaf length L, and leaf width W: $SA = a \times L \times W$.

A3.3 Animal model autocorrelation

Table A3.1 Autocorrelation of the naïve and maternal effects animal models for 2013 and 2014 data. Values represent the autocorrelation at lag 1 for each parameter.

		Naïve model		Maternal effects model		
Trait		**V_A**	**V_R**	**V_A**	**V_M**	**V_R**
Len	2013	0.006	0.006	0.005	-0.037	-0.010
	2014	-0.004	-0.003	0.038	-0.040	-0.027
Wid	2013	0.073	-0.012	0.068	0.080	-0.030
	2014	-0.022	-0.016	0.084	0.083	-0.004
SA	2013	0.020	0.012	0.055	-0.029	0.021
	2014	-0.007	0.019	0.041	-0.029	0.017
Wm	2013	-0.022	0.030	0.009	0.013	0.019
	2014	-0.011	0.011	0.015	0.031	-0.001
Dm	2013	-0.008	0.039	0.023	0.009	-0.014
	2014	-0.019	-0.035	0.008	-0.001	-0.057
TD	2013	-0.041	-0.016	0.034	-0.008	0.024
	2014	-0.024	0.006	0.078	-0.009	0.000
SLA	2013	-0.006	0.012	0.032	-0.015	-0.034
	2014	-0.014	-0.057	0.016	-0.015	-0.017
Til	2013	0.018	-0.007	-0.009	0.030	-0.021
	2014	0.017	0.008	0.005	0.022	-0.053
Bio	2013	0.018	0.016	-0.030	-0.019	-0.051
	2014	-0.047	-0.026	-0.001	0.003	-0.094
TFlw‡		0.038	-0.025	0.031	-0.011	-0.024
TSeed‡		0.009	-0.005	0.016	-0.035	0.049

Len = leaf length; Wid = leaf width; SA = leaf surface area; TD = tissue density; SLA = specific leaf area; Til = number of tillers in September; Bio = biomass above 25 mm; TFlw = total number of flowers summed across 3 years; TSeed = total number of seeds summed across 3 years. ‡ These are the values summed across three years, 2013, 2014 and 2015, and results taken from comparisons with 2013 trait data.

A3.4 Table of families for each trait for parent-offspring regression heritability estimates

Table A3.2 The number of different families of each size analysed in the parent-offspring regression analysis for each trait.

Family	2013									2014									Sum	
size	Len	Wid	SA	Ww	Dw	TD	SLA	Til	Bio	Len	Wid	SA	Ww	Dw	TD	SLA	Til	Bio	TFlw	TSeed
1	199	199	199	199	199	199	199	199	199	196	196	196	186	196	186	196	199	199	192	188
2	45	45	45	45	45	45	45	45	45	45	45	45	42	45	42	45	45	45	44	42
3	13	13	13	13	13	13	13	13	13	13	13	13	11	13	11	13	13	13	12	11
4	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
10	1	1	1	1	1	1	1	1	1	1	1	1	-	1	-	1	1	1	1	1
Total	267	267	267	267	267	267	267	267	267	264	264	264	248	264	248	264	267	267	258	251

Len = leaf length; Wid = leaf width; SA = leaf surface area; TD = Tissue density; SLA = Specific leaf area; Ww = Weg weight; Dw = Dry weight; Til = Number of tillers in September; Bio = Biomass above 25 mm; TFlw = Total number of flowers summed across 3 years; TSeed = Total number of seeds summed across 3 years.

A3.5 Flowering summary

Table A3.3 A summary of the *F. ovina* flowering during the course of the common garden experiment.

No. flowering tillers	2013	2014	2015	TFlw
0	287	232	339	131
1	182	170	120	122
2	33	84	39	108
3	2	18	6	79
4	NA	NA	NA	38
5	NA	NA	NA	21
6	NA	NA	NA	5

TFlw = total number of flowers summed across 3 years

A3.6 Animal model variance components

Table A3.4 Breakdown of the variance components for each model.

		Naïve model		Maternal effects model		
Trait		V_A	V_R	V_A	V_M	V_R
Len	2013	0.073	0.077	0.078	0.191	0.078
	2014	0.069	0.086	0.081	0.181	0.086
Wid	2013	0.061	0.049	0.066	0.182	0.053
	2014	0.061	0.051	0.068	0.183	0.058
SA	2013	0.297	0.699	0.367	0.301	0.602
	2014	0.406	0.857	0.381	0.323	0.911
Wm	2013	0.110	0.190	0.142	0.212	0.166
	2014	0.135	0.219	0.142	0.215	0.209
Dm	2013	0.080	0.105	0.090	0.188	0.097
	2014	0.099	0.134	0.099	0.191	0.126
TD	2013	0.073	0.064	0.083	0.195	0.071
	2014	0.111	0.108	0.123	0.209	0.111
SLA	2013	0.080	0.071	0.087	0.176	0.074
	2014	0.082	0.074	0.094	0.203	0.079
Til	2013	0.118	0.107	0.119	0.214	0.105
	2014	0.129	0.118	0.129	0.193	0.122
Bio	2013	0.394	0.916	0.480	0.387	0.616
	2014	0.384	0.691	0.443	0.329	0.693
TFlw‡		0.593	1.046	0.610	0.406	1.086
TSeed‡		1.457	3.090	1.486	0.687	2.899

Len = leaf length; Wid = leaf width; SA = leaf surface area; TD = tissue density; SLA = specific leaf area; Til = number of tillers in September; Bio = biomass above 25 mm; TFlw = total number of flowers summed across 3 years; TSeed = total number of seeds summed across 3 years. ‡ These are the values summed across three years, 2013, 2014 and 2015, and results taken from comparisons with 2013 trait data.

A4 Appendix 4

A4.1 Chromosome counting methods

Chromosome counts were carried out by Dr Hugh McAllister. The individual with the largest and smallest genome size in the main range of variation (as measured using flow cytometry), along with individuals with outlier genome size measurements, were selected for chromosome counting, for parents and offspring.

Chromosome counts were conducted using a method modified from Dyer (1963). Chromosome counts were conducted on rapidly growing root tips. Root tips of ~1 cm length are removed with forceps and placed in vials of 1-bromo-naphthalein solution at room temperature for four hours. Vials were placed in a fridge overnight at ~1°C. Root tips were fixed in a solution of 3:1 ethanol (95%): glacial acetic acid for 12 hours at –18°C. Root tips were removed from the vials and transferred into preheated vials of 1 M HCl at 60°C for 5 minutes. After hydrolysis, root tips were placed in 70% ethanol and stored for at least 12 hours. The root tip was then placed in a drop of 70% ethanol on the edge of a slide and examined under a dissecting microscope. The root cap was removed using fine needles and the densely cytoplasmic region removed. This was placed in drop of mounting solution of 2:1 lactic acid: propionic acid, in the centre of the slide and a coverslip added. The slide was tapped with a needle to produce a monolayer of cells. The slide was then blotted to remove excess mounting solution and squashed between absorbent paper. The preparation was examined using phase contrast microscopy.

A4.2 Genome size measurement repeatability

Each genome size measurement is an average of three measurements of genome size for each plant. The repeatability of the genome size measurements was calculated using a linear mixed effects model in the statistical software R (R Development Core Team 2008), in the package LMER (Bates *et al.* 2015). The dataset consisted of each of the three genome size measurements for each clonal line. Clone was fitted as a random effect. This apportioned the variance in genome size into that which was within clonal line, and that which was among clonal line. The repeatability was then calculated as:

$$R = \frac{Between\ clone\ variance}{Total\ variance},$$

following (Nakagawa & Schielzeth 2010). The repeatability of genome size measurement was R = 0.963, which decreased to R = 0.702 when measurements for the outlier individual 2936 were removed from the dataset.

A5 Appendix 5

A5.1 Selection of *F. ovina* clonal lines

We used 48 clonal lines of *F. ovina* in the microcosm community experiment. The material came from the *offspring clonal library,* the collection of F1 progeny of plants collected from BCCIL. The 48 clonal lines were selected based on their maternal parents' climate treatment plot and soil depth at BCCIL. This proceeded as follows.

First, using a random stratified process, we selected 24 of the 59 parent plants collected from BCCIL. The 24 parents were chosen so that 6 parents were selected from the joint category of climate treatment × soil depth, resulting in 4 variables: control, deep; control, shallow; drought, deep; and drought, shallow. Soil depth was measured continuously at BCCIL, but values were then split into a binary category of shallow or deep soil. Where possible, each plot block at BCCIL (A-E) was represented within each climate and soil depth combination.

Second, for each of the 24 parent plants identified, we then selected at random (using a random number generator) 2 offspring individuals. This resulted in a total of 48 clonal lines, selected from 24 maternal parents. When planting the clonal lines, if a selected plant did not have enough tillers to split for the required number of replicates, then a new offspring individual was selected at random for the same maternal parent. The 48 clonal lines spanned the known range of trait variation within the experimental population.

A5.2 Environmental monitoring

Soil moisture was monitored during the drought treatment with a soil moisture probe (Theta Probe ML2x, Delta-T Devices Ltd). Soil moisture readings were taken in mV and then transformed to volumetric water content by:

$$w = \frac{1.07+6.4m-6.4m^2+4.7m^3-a_0}{a_1}$$

where w = volumetric water content, measured in kg.l^{-1}, m = soil moisture reading in volts, and a_0 and a_1 are parameters specific to the soil mix used.

To calculate a_0 and a_1, three replicated pots containing the soil mixture used in the microcosms were made. The pots were watered to saturation and then dried in an oven for one week, measuring the volume and mass after each process. In what follows, m refers to soil moisture readings in volts, M to measured mass of soil in kilograms, and V to the volume of soil in litres. The subscripts s and d refer to saturated and dried soil respectively.

The parameters a_0 and a_1 were then calculated as follows:

$$a_0 = 1.07 + 6.4m_d - 6.4{m_d}^2 + 4.7{m_d}^3$$

and

$$a_1 = \frac{1.07 + 6.4m_s - 6.4{m_s}^2 + 4.7{m_s}^3 - a_0}{z}$$

where $z = \frac{M_s - M_d}{V}$.

For this soil mixture a_0 and a_1 were calculated to be a_0 = 1.525 and a_1 = 10.409

A5.3 Biomass calibration

In order to determine the correlation between biomass above 25 mm with total plant vegetative biomass, 18 microcosms were set up following identical methods to those detailed in Chapter 5, using two clones of *F. ovina* and a single clone of each of *K. macrantha*, *C. panicea* and *C. caryophyllea*. During the course of the experiment these microcosms followed identical management to the microcosms in the experiment experiencing ambient conditions. In September 2015 the plants in these pots were sampled destructively, first collecting biomass above 25 mm and then the remaining above soil vegetative biomass. Biomass clippings were dried for 1 week at 55 °C and then weighed.

The correlation between the biomass above 25 mm and the total vegetative biomass were analysed for each species. Data for *F. ovina* were analysed with a linear mixed effects model in the R package LMER (Bates *et al.* 2015), with a Gaussian family, and with clone fitted as a random effect. For the LMER model R^2 was calculated following Nakagawa (2013), and both marginal and conditional R^2 is reported. *K. macrantha, C. panicea* and *C. caryophyllea* data were analysed with a linear model with a Gaussian family.

There was a strong correlation between the vegetative biomass above 25 mm and the total vegetative biomass for each of the four species under study. *F. ovina*, conditional R^2 (includes random effects) = 0.82, marginal R^2 = 0.75 (does not include random effects), $p < 0.001$; *K. macrantha*, $R^2 = 0.45$, $p < 0.01$; *C. caryophyllea*, $R^2 = 0.78$, $p < 0.001$; *C. panicea*, $R^2 = 0.85$, $p < 0.001$ (Figure A5.1).

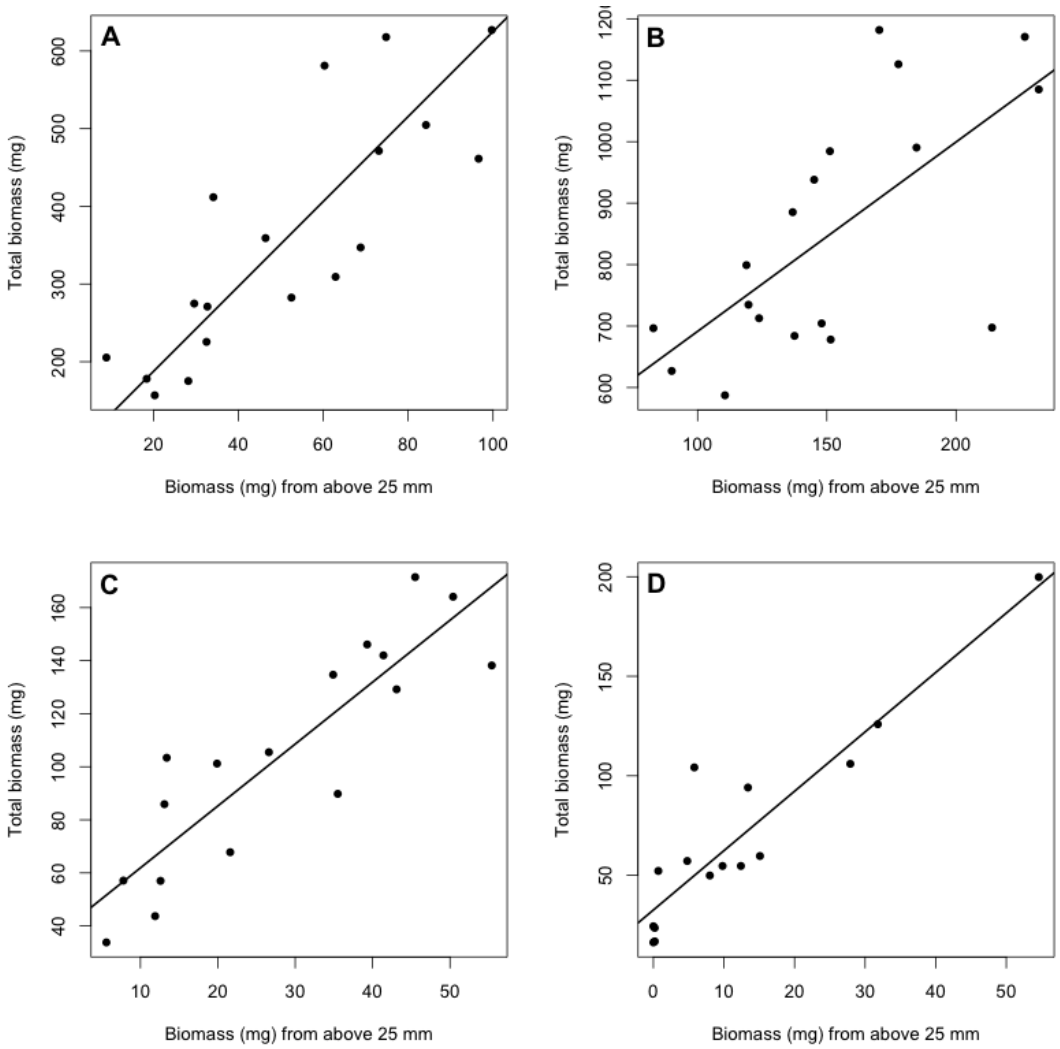

Figure A5.1 The correlation between biomass mg above 25 mm and the total plant above ground biomass mg for **A)** *Festuca ovina,* **B)** *Koleria macrantha*, **C)** *Carex caryophyllea* and **D)** *Carex panicea*.

A5.4 Recovery from drought

The recovery of *F. ovina* following the drought treatment was measured by a biomass clipping at 25 mm in October 2015: this meant any new growth had grown within the month since the biomass clipping that immediately followed the end of the drought (September 2015). The re-growth biomass was analysed as a proportion of growth from the biomass below 25 mm. Although the quantity of

biomass below 25 mm was not measured directly this value was predicted from the September biomass clipping using the following equation:

$$B_L = 4.569\ B_S + 78$$

where B_S = biomass above 25 mm from the September 2015 clipping in mg and B_L = predicted vegetative biomass below 25 mm in mg.

The constants 4.569 and 78 were calculated from the biomass calibration.

The proportion of re-growth biomass was then calculated as:

$$\text{Proportion re-growth biomass} = B_R/B_L$$

where B_R = Regrowth biomass (October 2015 clipping) in mg.

A5.5 Total living canopy surface

We used photographs of the microcosms to monitor the senescence of the canopy of *F. ovina* during the drought. Photographs of the microcosms were taken at the start, middle, and end of the drought, from a standard height above each microcosm using a digital camera (Cannon ESO 1000D). Photos were taken using the macro setting with manual focus. Images were analysed in a point quadrat type manner using the GRIDS plugin in ImageJ (Abramoff, Magalhaes & Ram 2004). A crosses type grid was used with an area per point of 12000 pixels^2. For each

photograph, the image detail under the intersection of a cross was examined to determine whether there was contact with *F. ovina* tissue, and if so, whether this was dead or alive (Figure A5.2). Contacts were tallied giving a value of the total contacts with living tissue of *F. ovina* as a proportion of total contacts with leaf tissue of *F. ovina*.

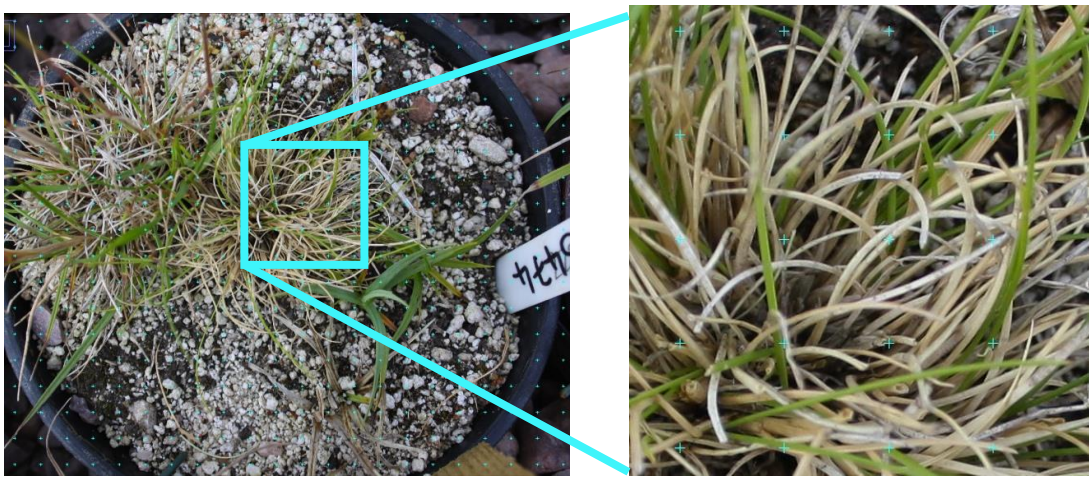

Figure A5.2 Analysis of senescence in *Festuca ovina* was measured using photographs of the microcosms. The above image shows an example photograph (left) and a close up (right) of the image with the crosses that were used to implement a "point quadrat" style analysis. The status of contacts with *F. ovina* under each cross were recorded as dead or alive.

A5.6 Outlier removal for bivariate response models

Two microcosms were identified as outliers and excluded from the bivariate response model analysis because they had excess vegetative biomass growth. One microcosm had excessive growth of vegetative biomass for both species of *Carex*. The biomass produced in this microcosm by *C. caryophyllea* was 259.7 mg, which lay more than 12 standard deviations away from the mean (mean = 13. 1 mg; s.d. =

19.3). The biomass produced in this microcosm by *C. panicea* was 206.70 mg, which lay more than 8 standard deviations away from the mean (mean = 17.9 mg; s.d. = 21.3). Therefore the data from this microcosm was classed as an outlier and excluded from this set of analyses. A second microcosm in which the biomass produced by *C. panicea* was 214.6 mg, which lay more than 9 standard deviations away from the mean, was also classed as an outlier and removed from this set of analyses. This reduced the total number of microcosms under study to 274.

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